MAY 8 1944

Volume 34 Number 4

Phytopathology

AN INTERNATIONAL JOURNAL

April 1944

Official Organ of

The American Phytopathological Society

133

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere

Office of Publication

Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania

Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter

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Subscription price. $6.00 per year United States and dependencies; Canada, $6.25; other countries $6.50; single numbers of the current volume, 60 cents each. The journal has been issued monthly beginning with Volume VIII, January, 1918. Prior to that it was issued bimonthly.

Back Volumes VIII to XXXII, inclusive (except Vol. VIII, Nos. 1 and 8, Vol. IX, No. 1, and Vol. X, No. 1, which are not available) unbound at $8.00 per volume. All earlier volumes obtainable only in broken numbers.

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Requests to supply copies of the journal must be made within 60 days from date of issue. Date of issue January 15 and monthly thereafter.

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1909–1943

L. A. Alvarez Garcia

Andrés R. López, for seven years Assistant Plant Pathologist of the Agricultural Experiment Station of the University of Puerto Rico and a member of the American Phytopathological Society, died on February 4, 1943, after an illness of one and a half years.

Mr. López, first son of Mr. Ramón López Linares and Mrs. Teresa Elías de Linares, was born on February 4, 1909, on a coffee farm near San Sebastián, Puerto Rico, and attended elementary school in that town. He entered the High School Course of the Polytechnic Institute, at San Germán, P. R., where he was graduated in the year 1928. In the same year he entered the College of Agriculture and Mechanic Arts of the University of Puerto Rico, where he began the general course in agriculture. At the end of his first year he enrolled at Louisiana State University, where he received the degree of Bachelor of Science in Agriculture in 1933. Continuing his post-graduate studies, he was awarded in 1935 his M.S. degree, with a major in Plant Pathology. While at Louisiana he had opportunity to acquaint himself with the different types of sugar-cane mosaics under study at the University Experiment Station. This experience later proved of value in his work and in the preparation of his unpublished thesis on mosaic resistance of different cane varieties.

Upon his return to Puerto Rico he started work as Agronomist in the Federal Emergency Relief Administration, and, later, taught vocational agriculture at one of the Second Unit Schools of the Department of Education at San Sebastián, Puerto Rico. He joined the technical staff of the Experiment Station of the University of Puerto Rico in 1936 as Assistant Plant Pathologist, thus coming to work in the particular line of studies that had been his chosen vocation. Greatly interested in his work, he attended Iowa State University during 1939–40, where he pursued further phytopathological studies.

Shortly after resuming his duties at the Insular Experiment Station he married Miss Belén H. Cestero, from Vega Baja, Puerto Rico, and but a few months later was taken seriously ill from a childhood infection of Bilharzia from which he never recovered.

With his death both the agricultural experiment station and the agriculture of the Island lose a genuine scientist of great promise, and his colleagues a true and sincere friend.

STATISTICAL STUDIES OF DISTRIBUTION OF PSOROSIS-AFFECTED TREES IN CITRUS ORCHARDS[1]

A. A. BITANCOURT[2] AND H. S. FAWCETT[3, 4]

(Accepted for publication December 15, 1943)

INTRODUCTION

In a number of citrus orchards in California, careful records of psorosis-affected trees have been kept by orchard managers and tree surgeons. These records, as represented on maps on which the diseased trees have been charted, furnish excellent material for statistical analysis. This analysis has thrown light on the distribution and probable manner of spread of this disease in orchards.

As shown by Fawcett (3, 4), psorosis of citrus is a virosis that manifests itself by leaf symptoms of the mosaic type and by bark lesions. On young leaves both the veinlets and the adjacent tissue show faint to pronounced clearing. These small cleared areas, about 1 to 2 mm. long and ½ to 1 mm. broad, may be numerous and scattered over the entire leaf blade, or they may be limited to certain portions. Frequently, a distinct pattern, such as the well-known "oak-leaf pattern," is formed. These cleared areas usually disappear as the leaf matures. Bark symptoms rarely occur on trees less than 6 to 8 years of age. They usually begin either as scales of bark or as aggregations of small pustules, under which are brown specks, on the surface of the older bark of trunks or limbs. As the scaling advances, the deeper layers of bark, and eventually the wood, become affected. A considerable amount of gum occurs near the cambium and between layers of wood, some of which is pushed to the surface (7). The wood in later stages becomes light drab to brown or reddish brown, the stain progressing in an irregular fashion, not necessarily following the grain of the wood (2). Although the leaf symptoms have considerable importance in connection with the study of the disease and are of practical importance for the identification of diseased trees, the bark symptoms are the only ones that are readily recognized. The records used for the present studies were, accordingly, those of diseased trees already showing bark symptoms.

[1] Paper No. 501, University of California Citrus Experiment Station, Riverside, California.

Part of this paper was presented at the twenty-sixth annual meeting of the Pacific Division of The American Phytopathological Society, Salt Lake City, Utah, June 17 to 19, 1942 (1). The work reported in this paper was begun during the tenure, by the senior writer, of a John Simon Guggenheim Memorial Foundation Fellowship for research at the University of California Citrus Experiment Station, Riverside, in 1942, and was completed in 1943.

[2] Plant Pathologist, Instituto Biologico, São Paulo, Brazil.

[3] Professor of Plant Pathology and Plant Pathologist in the Experiment Station, University of California.

[4] The writers wish to express their appreciation to Messrs. P. S. Sloop, O. W. Murray, Homer Barnes, J. C. Perry, and T. A. Lombard for supplying maps and careful records of the distribution of psorosis-affected trees in the orchards under their management; and to Dr. E. R. Parker and Dr. F. M. Turrell for their criticism of the manuscript.

Observational and experimental evidence have shown that the transmission of psorosis is mostly due to the use of buds from diseased parents. Based on this knowledge, a system of registering healthy parent trees has been established in California. Trees thought to be healthy are tested according to certain rules involving the observation of both leaf and bark symptoms, and, if the test indicates that they are healthy, they are registered by the State Department of Agriculture. The propagator is then given evidence of registration, which may be shown to the buyers of trees budded from the registered parents (5, 6).

In the past few years, however, there has been strong indication that bud transmission alone could not account for all the psorosis-affected trees ob-

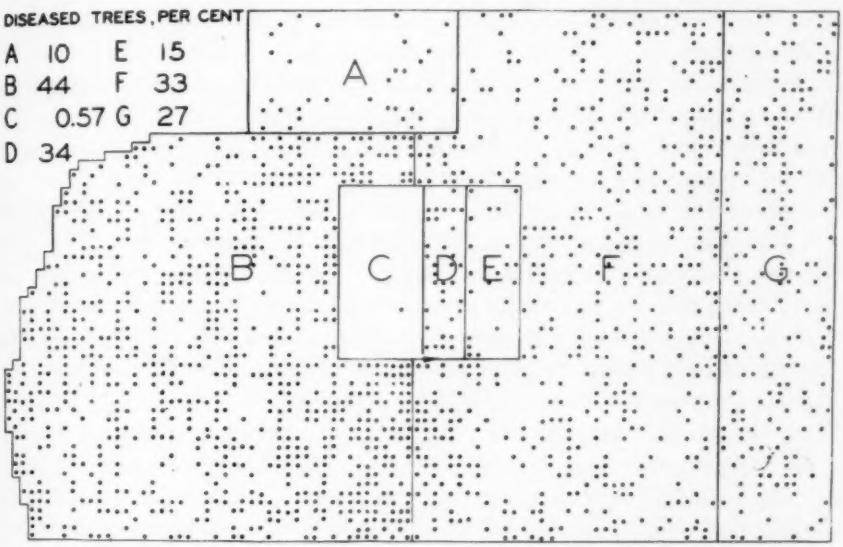

FIG. 1. Types of stencils used in counting diseased trees around diseased trees and diseased trees around healthy trees at distances L (A), 1.4 L (B), 2 L (C), and 2.8 L (D).

served in some orchards. For example, in a 35-year-old Valencia orchard in Orange County, 9 out of 10 new cases reported in 1939 developed on supposedly healthy trees that were adjacent to one or more diseased trees. In another orchard in Orange County, a planting of about 500 trees, the buds of which originated from 1 healthy tree, 4 trees have developed bark symptoms. In an orchard in Ventura County, where the origin of each tree that has been planted in the last 18 years has been carefully recorded, a small percentage (about 0.3 per cent) of trees budded from trees still living and that do not show any symptoms of psorosis, have developed the bark symptoms during the past few years. Similar evidence has been found in several other orchards.

In the majority of orchards in California, the trees of each orchard have originated from buds from a number of parent trees. Since in recent years

only has it been known that psorosis is caused by a virus transmitted by budding, a considerable number of younger trees with psorosis virus in them, but showing no bark symptoms, were used unwittingly by nurserymen as bud-parent trees. The percentage of psorosis-affected trees will vary from one planting to another, according to the relative numbers of diseased trees furnishing buds for propagation. When whole sections of a given orchard are composed of trees from different sources, very striking differences are observed, which can only be accounted for when those sources are known. An example is the orchard represented by the map in figure 1, 7 sections of which are known to have come from 7 different sources. The percentages of psorosis-affected trees in these sections were found to be 0.57, 10.0, 15.0, 27.0, 33.0, 34.0, and 44.0, respectively. The statistical method used in this paper cannot be applied to such heterogeneous orchards as a whole.

If the trees of an orchard come from different parent trees, some of which are healthy and some of which have psorosis, the conditions of propagation and planting in the orchard may have been such as to completely randomize the position of the diseased trees. This is probably the case in most orchards. The bud sticks usually were gathered by the nurserymen from several parent trees, with and without the virus in them. The sticks were tied into a bundle, with no attempt to keep separate the sticks from different parents. The sticks were further shuffled when the bundle was untied in the nursery and sticks were picked, one after another, in the process of budding the trees. As a result, whereas there was a certain amount of grouping in the nursery, owing to the fact that usually all the buds from the same stick were budded in a continuous series of trees, the sticks themselves were already more or less randomized when budding was performed. In the operations of digging out the trees, carrying them to the orchard, and planting them, there occurred several new opportunities for further randomization, so that the final position of the diseased trees in the orchard was random.

While such a random distribution of the diseased trees would remain the same in the absence of contamination of healthy trees by diseased trees, with spread of the disease there would be an increase in the number of diseased trees around the originally diseased trees, which would be determined by statistical analysis.

MATERIALS AND METHODS

The present study was based on the distribution of psorosis-affected trees, as recorded on maps of 14 orchards in Ventura, Orange, Riverside, and San Bernardino counties. The trees in these orchards were of miscellaneous parentage, and the percentages of diseased trees ranged from 4.8 to 70.6. Statistical methods were used to disclose possible grouping of trees that could only be accounted for by the transmission of psorosis from the diseased trees to the healthy trees in an orchard.

Most of the maps represented rectangular orchards, or rectangular parts of irregular orchard maps. Orchard 5 (Table 1) was made up of 2 sections,

TABLE 1.—*Mean incidence of psorosis-affected trees at distance L from central trees,[a] determined on maps of the various orchards*

Orchard No.	Age of trees, in years	Total number of trees	Percentage of diseased trees[b]	Calculations based on:									
				Four trees			Two trees						
							Along rows			Along arrays			Difference,[c] a and b
				Diseased	Healthy	Difference[c]	Diseased (a)	Healthy	Difference[c]	Diseased (b)	Healthy	Difference[c]	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	15	2159	4.8	0.174	0.189	−0.015	.....	.....	.....	.....	.....	.....	.....
2	50	1422	7.6	0.307	0.293	0.014	.....	.....	.....	.....	.....	.....	.....
3	16	1419	10.2	0.408	0.402	0.006	.....	.....	.....	.....	.....	.....	.....
4	20	2756	10.3	0.570	0.395	0.175**	0.357	0.190	0.167**	0.213	0.206	0.007	0.144**
5	22	2753	13.8	0.633	0.540	0.093*	0.292	0.273	0.019	0.341	0.264	0.077**	−0.049
6	33	911	14.9	1.193	0.498	0.695**	0.568	0.252	0.316**	0.633	0.246	0.387**	−0.065
7	32	1430	18.7	0.848	0.713	0.135*	0.408	0.358	0.050	0.450	0.345	0.105*	−0.042
8	29	702	32.0	1.484	1.181	0.303**	0.703	0.615	0.088	0.781	0.566	0.215**	−0.078
9	29	648	35.6	1.648	1.260	0.388**	0.791	0.650	0.141*	0.852	0.613	0.239**	−0.061
10	29	702	39.6	1.887	1.387	0.500**	0.895	0.738	0.157**	0.992	0.660	0.332**	−0.097
11	21	946	42.8	1.869	1.586	0.283**	0.952	0.789	0.163**	0.923	0.797	0.126**	0.029
12	29	675	52.5	2.427	1.747	0.680**	1.192	0.883	0.309**	1.235	0.875	0.360**	−0.043
13	32	570	61.6	2.577	2.251	0.326**	1.297	1.120	0.177**	1.280	1.136	0.144**	0.017
14	29	1029	70.6	2.924	2.554	0.370**	1.504	1.195	0.309**	1.422	1.359	0.063	0.082*

[a] For explanation of "central" trees and procedure used, see "Materials and Methods."
[b] Based on the number of central trees.
[c] * = significant (5 per cent level); ** = highly significant (1 per cent level).

1 rectangular and 1 slightly irregular; orchard 7 was made up of 4 small sections; orchard 6 of 2 sections, 1 rectangular and 1 irregular, of which only the rectangular part was used. Maps 8, 9, 10, and 12 represented 4 almost square blocks of a single orchard. They, however, showed different percentages of diseased trees and were, therefore, treated separately. The number of trees in the orchards ranged from 570, in orchard 13, to 2756, in orchard 4 (Table 1).

In orchards planted in squares (the only type used in the present investigations), there are 8 trees adjacent to every tree (Fig. 3), except those in

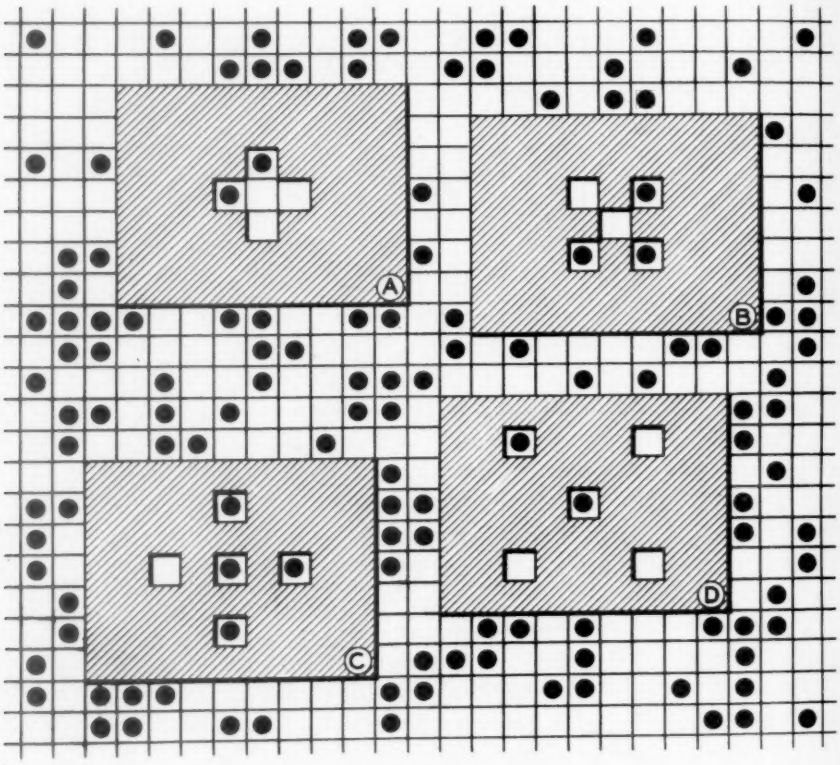

FIG. 2. Map showing distribution of psorosis-affected trees (black dots) in an orchard in Ventura County, California. The irregularities in the percentages of diseased trees in various sections are due to the fact that the buds of these trees came from seven different sources.

the borders. If the distance of each of the 4 trees closest to the central tree is L, then the distance of the 4 other trees is $L\sqrt{2}$, or approximately 1.4 L. Next to these 8 adjacent trees are 16 trees, of which only 8 have been considered in the present study, that is, those at a distance 2 L and 2.8 L from the central tree.

It seems reasonable to assume that if a central tree is diseased and has contaminated some of the adjacent trees, the number of diseased trees around it would be greater than around a central healthy tree.

The counting of diseased trees around diseased trees and of diseased trees around healthy trees was made on the maps with the aid of appropriate stencils (Fig. 2). Each stencil, cut from a piece of paper, disclosed 5 trees, namely, the central tree and 4 others at a given distance (L, 1.4 L, 2 L, or 2.8 L). On the tally sheet, there were, for each of these distances, 2 columns, 1 for diseased trees around diseased central trees and 1 for diseased trees around healthy central trees. In figure 2, A, for example, it may be seen that the central tree is healthy, and that there are 2 healthy trees and 2 diseased trees at distances L from the central tree. Accordingly, in this case, the entry on the tally sheet, under "distance L, healthy central trees," was "2." The stencil was then moved on to the next tree in the row, and the reading for diseased trees was made and entered on the tally sheet, and so on, until readings had been recorded for all the trees available for this stencil. The frequencies were then summed up, and the means of the two columns under "distance L," their difference, and its error were calculated. Similar readings and calculations were made for each of the other distances, 1.4 L, 2 L, and 2.8 L.

RESULTS

For each of the 14 orchards, the mean incidence of psorosis-affected trees adjacent to diseased and to healthy trees, and their mean differences, at the various distances, are shown in tables 1 and 2.[5]

The percentages of diseased trees shown in tables 1 and 2 are based on the number of central trees used in each calculation and are slightly different for different distances. While most of the trees in the orchards, in applying any given stencil, were used once as central trees and 4 times as trees at the given distance from central trees, certain border trees in this calculation were not used as central trees because they did not have 4 trees around them at that distance. The diseased trees in these border rows, not used as central trees for a given distance, were not counted for the total percentage of diseased trees for that distance. Border trees (in any rectangular orchard) were used from 1 to 3 times, both at distance 1.4 L and at distance 2.8 L from central trees, according to position. At distance L, however, there is 1 tree at each corner, and at distance 2 L there are 4 trees at each corner, not used at all. Whereas there are only 2 rows of border trees that are used less than 4 times around central trees at distances L and 1.4 L from central trees, there are 4 rows at distances 2 L and 2.8 L.

The "t" test was used to ascertain the significance of the differences (8). In the tables, highly significant differences corresponding to a probability of 1 per cent, or less, are marked with two asterisks; significant differences, between the 5 and 1 per cent levels, are marked with one asterisk. It may be seen in table 1 that, for orchard 1, the mean number of diseased trees is slightly, but not significantly, larger around healthy trees than around diseased trees. All other orchards show a greater mean number of diseased

[5] All calculations were made with a slide rule, and the means are affected with errors of not more than 0.2 per cent.

TABLE 2.—*Mean incidence of psorosis in trees at distances 1.4 L, 2 L, and 2.8 L from diseased and healthy central trees,[a] determined on maps of the various orchards*

Orchard No.	1.4 L				2 L				2.8 L			
	Percentage of diseased trees[b]	Mean incidence			Percentage of diseased trees[b]	Mean incidence			Percentage of diseased trees[b]	Mean incidence		
		Diseased trees	Healthy trees	Difference[c]		Diseased trees	Healthy trees	Difference[c]		Diseased trees	Healthy trees	Difference[c]
4	10.3	0.407	0.415	−0.008	10.3	0.476	0.416	0.060	10.3	0.480	0.420	0.060
5	13.9	0.623	0.533	0.090*	13.8	0.643	0.525	0.118**	13.7	0.575	0.531	0.044
6	14.9	1.211	0.503	0.708**	15.6	0.922	0.548	0.374**	15.6	0.798	0.550	0.248**
7	18.7	0.758	0.715	0.043	18.8	0.702	0.727	−0.025	18.8	0.739	0.717	0.022
8	32.0	1.349	1.248	0.101	31.2	1.329	1.244	0.085	31.2	1.266	1.302	−0.036
9	35.6	1.597	1.249	0.348**	34.6	1.610	1.263	0.347**	34.6	1.283	1.419	−0.136
10	39.6	1.731	1.508	0.223**	39.6	1.740	1.451	0.289**	39.6	1.620	1.490	0.130
11	42.8	1.906	1.554	0.352**	43.3	1.796	1.668	0.128	43.3	1.734	1.651	0.083
12	52.5	2.285	1.930	0.355**	52.4	2.297	1.926	0.371**	52.4	2.225	2.039	0.186
13	61.6	2.512	2.350	0.162	62.6	2.512	2.322	0.190	62.6	2.418	2.336	0.082
14	70.6	2.877	2.638	0.239**	70.2	2.906	2.539	0.367**	70.2	2.863	2.645	0.218**

[a] For explanation of "central" trees and procedure used, see "Materials and Methods."
[b] Based on the number of central trees.
[c] * = significant (5 per cent level); ** = highly significant (1 per cent level).

trees at distance L from diseased trees than at distance L from healthy trees. Except for orchards 2 and 3, the differences found are all significant or highly significant statistically.

Substantially the same results were obtained at the 3 other distances (Table 2). Orchards 1, 2, and 3 were not treated, but results for the other orchards show that the numbers of diseased trees around diseased trees as centers are usually significantly greater statistically than the numbers of diseased trees around healthy trees as centers. Negative differences were observed, however, at distance 1.4 L for orchard 4, at distance 2 L for orchard 7, and at distance 2.8 L for orchards 8 and 9. Furthermore, differences were statistically significant, or highly significant, in only 7 of the 11 orchards at distance 1.4 L, in only 6 orchards at distance 2 L, and in only 3 at distance 2.8 L. The tables also show that there is usually a marked decrease in the differences as the distance increases. The means of all the differences, and their standard errors, not counting the negative differences, are 0.305 ± 0.022, for 13 orchards at distance L; 0.262 ± 0.025 and 0.233 ± 0.027, for 10 orchards at distances 1.4 L and 2 L, respectively; and 0.119 ± 0.027, for 9 orchards at distance 2.8 L.

The decrease of the differences, per unit distance L (assuming that the difference at central tree is 1), is 0.695 between the central trees and the trees at distance L; 0.108 between trees at distances L and 1.4 L; 0.049 between trees at distances 1.4 L and 2 L; and 0.142 between those at distances 2 L and 2.8 L. As will be seen later, this decrease per unit distance from the central tree may represent a mean gradient of infectivity.

GROUPING OF DISEASED TREES ALONG ROWS OF PLANTING

The method that has just been described therefore discloses a grouping of diseased trees.[6] Such a grouping of trees might be due to the fact that trees originating from the same diseased parents were kept together during the several operations from the budding in the nursery to the planting in the orchard. It is easily seen that grouping due to such a cause would increase the differences in the numbers of diseased trees around diseased trees, and around healthy trees, only for the distances L and 2 L. For the distances 1.4 L and 2.8 L, that is, along the diagonals of the orchard, no such effect would be produced in planting. Furthermore, if the grouping of trees is due to this cause, it would take place along only one of the two general directions of rows of trees in the orchard, that is, along rows or along arrays, according to the direction in which the orchard was planted, and analysis as above, using the 2 trees at distance L from central trees along rows, and the 2 trees at distance L from central trees along arrays, separately, would disclose the fact. This analysis in the 2 directions at right angles to each other was made for all the orchards mentioned in table 1, except orchards 1,

[6] The same grouping also might be shown by fitting a theoretical random distribution, such as the binomial or the Poisson distributions, and applying the χ^2 test of goodness of fit. This method was used extensively in the course of the present study, but, inasmuch as it merely confirms the conclusions reached by the "t" test without giving a measure of the differences between diseased and healthy trees, the results obtained are not given here.

2, and 3, which in the previous analysis had not shown significant differences at distance L. The results have been entered in columns 8 to 13 of table 1. In column 14 the differences between the mean numbers of diseased trees at distance L from diseased trees, along rows and along arrays, have been entered.

It may be seen in table 1 that in orchards 6, 9, 10, 11, 12, and 13, there were significant or highly significant differences between the mean numbers of diseased trees around diseased trees and of diseased trees around healthy trees, both along rows (col. 10) and along arrays (col. 13). The differences between the numbers of diseased trees at distance L from diseased trees along rows and along arrays (col. 14) were not significant. It can be assumed that the diseased trees from the nursery were thoroughly randomized when planted in those orchards. No significant differences were found along rows in orchards 5, 7, and 8, or along arrays in orchards 4 and 14. Only in orchards 4 and 14, however, were there significant differences between the numbers of diseased trees on the 2 sides of diseased trees along rows and along arrays (col. 14). Only in those 2 orchards, therefore, is it safe to assume that there was grouping of diseased trees at the time of planting.

ANALYSES OF FICTITIOUS ORCHARDS COMPARED WITH THOSE OF ACTUAL ORCHARDS

As previously stated, the purpose of the present study was to disclose grouping of diseased trees that could be accounted for only by transmission of psorosis from diseased to healthy trees. In order to ascertain the effect of such transmission (to a known number of trees at different distances from given trees) on the differences calculated as described above, maps of fictitious orchards were prepared. Dots representing psorosis-affected trees, as in figure 2, were distributed on these maps according to the method described below.

Maps I, II, and III represented square orchards, map I with 50 rows of 50 trees each, and maps II and III with 33 rows of 33 trees each. Map IV had the same layout as orchard 11 (Tables 1 and 2), namely, 22 rows of 43 trees each. On each of these maps, a number of dots representing "diseased" trees were distributed by drawing at random, first the number of a row, from one set of numbered cards, and then the number of a tree, from another set. Counts and calculations were then made for distance L on all four maps, and also for the other three distances on map I (Table 3). In the next step, a given number of dots representing "contaminated" trees were placed at random at distance L from the other dots representing "diseased" trees. This was done by drawing at random, first the number of a row, then the number of a "diseased" tree, or dot, and then the direction, north, south, east, or west. Counts and calculations were then again made for distance L on maps I, II, and III. Finally, the same procedure was used for placing given numbers of dots ("contaminated" trees) at the other 3 distances (1.4 L, 2 L, and 2.8 L) from the "diseased" trees, and the same method of calculation was again applied.

TABLE 3.—*Data obtained from maps of fictitious orchards, on which given percentages of "contaminated" trees were placed at random at distances L, 1.4 L, 2 L, and 2.8 L from randomized "diseased" trees and analyzed as in tables 1 and 2*

Map and count Nos.	Distance from central tree	Percentage of contaminated trees	Percentage of diseased trees[a] (p)	Mean incidence			$\frac{pd}{4}$
				Diseased trees	Healthy trees	Difference[b] (d)	
1	2	3	4	5	6	7	8
Map I							
Count 1	L	0	8.03	0.270	0.325	−0.055	
	1.4 L	0	8.03	0.373	0.316	0.057	0.11
	2 L	0	7.99	0.355	0.315	0.040	0.08
	2.8 L	0	7.99	0.367	0.314	0.053	0.11
Total		0					0.30
Count 2	L	5	13.03	1.230	0.414	0.816**	2.66
Count 3	L	5	19.1	1.291	0.635	0.656**	3.13
	1.4 L	3	19.1	1.209	0.652	0.557**	2.64
	2 L	2	18.8	1.033	0.688	0.345**	1.62
	2.8 L	1	18.8	0.940	0.717	0.223**	1.05
Total		11					8.44
Map Ia							
	L	5	19.1	1.443	0.597	0.846**	4.03
	1.4 L	3	19.1	1.223	0.646	0.577**	2.76
	2 L	2	18.8	0.964	0.708	0.256**	1.20
	2.8 L	1	18.8	0.975	0.706	0.269**	1.23
Total		11					9.22
Map II							
Count 1	L	0	15.2	0.603	0.600	0.003	0.0
Count 2	L	9	24.2	1.474	0.793	0.681**	2.6
Count 3	L	9	32.7	1.653	1.122	0.531**	4.3
	1.4 L	5	32.7	1.538	1.161	0.377**	3.1
	2 L	3	32.2	1.522	1.179	0.343**	2.8
	2.8 L	0	32.2	1.296	1.286	0.010	0.1
Total		17					10.3
Map III							
Count 1	L	0	19.5	0.770	0.782	−0.012	.....
Count 2	L	15	34.4	1.866	1.125	0.741**	6.4
Count 3	L	15	54.7	2.409	1.893	0.516**	7.0
	1.4 L	10	54.7	2.278	2.044	0.234**	3.2
	2 L	5	54.8	2.396	1.906	0.490**	6.7
	2.8 L	3	54.8	2.226	2.081	0.145*	2.0
Total		33					18.9
Map IV							
Count 1	L	0.0	42.8	1.692	1.713	−0.021	.....
Count 3	L	13.8	77.3	3.125	2.967	0.158	3.1
	1.4 L	7.8	77.3	3.202	2.712	0.490**	9.5
	2 L	7.8	79.8	3.200	2.923	0.277**	5.5
	2.8 L	3.8	79.8	3.125	2.993	0.132	2.6
Total		33.2					26.7

[a] Based on the number of central trees.
[b] * = significant (5 per cent level); ** = highly significant (1 per cent level).

Map Ia was identical with map I, except that 50 per cent of the trees placed at distance 1.4 L from the diseased central trees were placed entirely at random, and 50 per cent were placed next to contaminated trees at distance L from the central trees, while the contaminated trees at distances 2 L and 2.8 L from central trees were so placed as to be adjacent to contaminated trees at distances L and 1.4 L, respectively, from central trees. This map, therefore, reproduces the condition of an orchard in which the disease would have been transmitted to trees at distances 2 L and 2.8 L from central trees indirectly through already contaminated trees at distances L and 1.4 L, respectively, from diseased central trees. Half the trees at 1.4 L from central trees presumably would have been contaminated directly by the diseased central trees, and half indirectly through contaminated trees at distance L from the central trees.

Results of analyses of maps of fictitious orchards are shown in table 3. It is seen that, as might have been expected, the first randomization (count 1) did not show significant differences between the mean numbers of diseased trees around diseased trees and around healthy trees. When, however, dots representing "contaminated" trees were placed at given distances from "diseased" trees, significant differences appeared.

Comparison of studies of maps I and Ia (Table 3) shows that, when "contamination" of trees at distances 1.4 L, 2 L, and 2.8 L is not the result of direct transmission from diseased central trees, the differences for distance L are increased. The same undoubtedly also applies to distance 1.4 L, but did not show in the case of maps I and Ia, owing to the comparatively large errors affecting these differences.

Comparison of the results from the fictitious orchards (Table 3) with those from the actual orchards (Tables 1 and 2) shows that map I was somewhat comparable to orchard 6, and map III to orchard 12 (Table 4). All the actual orchards showed smaller differences than the fictitious orchards, but not substantially so in several cases. Orchard 6 was the only one in which a very conspicuous grouping could be detected on the map. Grouping in the other orchards was not readily detected by simple examination of the maps.

DISCUSSION

From the results obtained with the fictitious orchards, it must be concluded that, assuming random distribution of diseased trees at the time of planting in an orchard, a later grouping of diseased trees, revealed by the method reported in this paper, indicates a subsequent transmission of disease to healthy trees. The method reveals groupings that cannot be detected readily by looking at a map.

If, instead of using the same trees once as central trees and several times as adjacent trees, each tree had been used only once, that is, if the orchard had been divided into groups of 5 trees, each group consisting of a central tree and the 4 adjacent trees at the given distance, the percentage of contaminated trees could have been calculated. We have seen that, in

TABLE 4.—*Differences and estimated percentages of contaminated trees of actual and fictitious orchards—orchard 6 compared with map I and orchard 12 compared with map III (see Tables 2 and 3)*

Distance	Orchard 6		Map I		Distance	Orchard 12		Map III	
	Difference	Percentage of contaminated trees	Difference	Percentage of contaminated trees		Difference	Percentage of contaminated trees	Difference	Percentage of contaminated trees
L	0.695	2.6	0.656	3.1	L	0.680	8.9	0.516	7.0
1.4 L	0.708	2.6	0.557	2.6	1.4 L	0.355	4.7	0.234	3.2
2 L	0.374	1.5	0.345	1.6	2 L	0.371	4.9	0.490	6.7
2.8 L	0.248	1.0	0.223	1.1	2.8 L	0.186	2.4	0.145	2.0
Total		7.7		8.4	Total		20.9		18.9

the absence of contamination, no significant difference was found between the mean number of diseased trees adjacent to diseased trees and the mean number of diseased trees adjacent to healthy trees. With due allowance for the effect of random errors, the difference, d, found when contaminated trees were added to the map, was, therefore, due to those trees. The probability of contamination of any one of the 4 adjacent trees by the central tree is, therefore, d/4, and if p represents the percentage of diseased central trees, pd/4 represents the percentage of trees contaminated by central trees at the given distance. However, inasmuch as the same trees are used as central trees and as adjacent trees, p and d are correlated, and the product is smaller than the actual percentage of contamination. In table 3 the determinations pd/4 have been entered in the last column and can be compared with the actual percentages of "contaminated" trees distributed on the maps. While there are minor variations owing to the comparatively large errors affecting the results, the formula in most cases gives results comparable to, although smaller than, the actual percentages of contamination.

The results reported for map Ia (Table 3, col. 8), for all distances except 2 L, are greater than those reported for map I. It is easily understood why the results for distance L, for instance, should be greater in map Ia, reproducing conditions of indirect transmission, than in map I, in which a perfect random distribution of contaminated trees at the 4 distances results in independent values for the 4 differences. In the case of map Ia, transmission of the disease from the central tree to a tree at distance 2 L is through the intermediate tree at distance L from both. The difference for distance L will, therefore, contain the effect of the transmission from the central tree to the tree at distance L and to the tree at distance 2 L; for the same reason, it will also contain the effect of transmission to a tree at distance 1.4 L. Similarly, the difference for distance 1.4 L will also contain the effect of transmission to distance 2.8 L. For maps I, II, III, and IV, an estimate of the total percentage of contaminated trees, therefore, is represented by the sum of the 4 calculated percentages (Table 3, col. 3), while such a sum is likely to be too great in cases like that of map Ia. The fact that the sum of the calculated percentages was 9.22, while the actual percentage was 11, is due to the fact that the estimates are too small, owing to the correlation between p and d and also to the effect of random errors. Apparently, a correct minimum estimate for such conditions as those represented by map Ia would be given by the sum of the estimates at distance L and at distance 2.8 L.

The results of the application of the formula pd/4 to the data of tables 1 and 2 are shown in table 5. These results are, of course, valid only in case there has been no other cause of grouping in the orchards. They are comparable to results that would be produced by percentages of transmission, at distance L, ranging from 0.3 in orchard 5 to 8.9 in orchard 12. These percentages correspond to 2.2 and 17 per cent of the total number of dis-

eased trees in the two orchards. If the mode of transmission to trees at distances 1.4 L, 2 L, and 2.8 L is direct, as in maps I, II, III, and IV, the figures in table 5 are independent and should be summed up to give a minimum estimate of the total percentage of contaminated trees at the 4 distances. This will give 1.2 per cent of contaminated trees for orchard 5, and 20.9 per cent for orchard 12, corresponding to 8.7 per cent, and 39.8 per cent, respectively, of the total number of diseased trees.

If, on the contrary, transmission is partially or totally indirect, as in the case of map Ia, the figures relative to distances L and 1.4 L also contain the effect of the transmission to trees at the other distances, and these sums are likely to be too great. The figure for distance L does not, however, contain the effect of transmission to distance 2.8 L, and the sum of the figures for these two distances is apparently a good minimum estimate of the percentage of transmission to the trees at the four distances, if no other cause of grouping occurs in the orchard. These totals range from 0.5 per cent in orchard 5 to 11.3 per cent in orchard 12, representing, respectively, 3.6 and 21.5 per cent of the total of diseased trees.

TABLE 5.—*Minimum estimates of the percentages of "contaminated" trees, calculated by means of the formula pd/4,*[a] *from the data in tables 1 and 2, on the assumption that "diseased" trees were randomized at time of planting*

Orchard No.	Percentage of diseased trees[b] (p)	Calculations (pd/4) at distances:			
		L	1.4 L	2 L	2.8 L
4	10.3	0.5	—[c]	0.2	0.2
5	13.8	0.3	0.3	0.4	0.2
6	14.9	2.6	2.6	1.5	1.0
7	18.7	0.6	0.2	—	0.1
8	32.0	2.4	0.8	0.7	—
9	35.6	3.5	3.1	3.0	—
10	39.6	5.0	2.2	2.9	1.3
11	42.8	3.0	3.8	1.4	0.9
12	52.5	8.9	4.7	4.9	2.4
13	61.6	5.0	2.5	3.0	1.3
14	70.6	6.5	4.2	6.4	3.8

[a] p = percentage of diseased trees; d/4 = probability of contamination of any one of 4 adjacent trees by the central tree (see "Discussion").

[b] Based on the number of central trees for distance L.

[c] Dashes indicate negative differences for d, where diseased trees were greater around healthy trees than around diseased trees.

Among the possible causes of grouping of psorosis-affected trees, other than the transmission of the disease from diseased to healthy trees, is, as already mentioned, the grouping along rows by the planting of trees from the same parent trees. This would not affect the grouping along the diagonals, detected in the analysis for trees at distances 1.4 L and 2.8 L. For instance, the estimates of 0.2 per cent at distance 2.8 L for orchard 4 and of 4.2 per cent and 3.8 per cent at distances 1.4 L and 2.8 L, respectively, for orchard 14 (Table 5), are entirely independent of a possible grouping of trees at the time of planting. It was shown that in the case of only two orchards (see Table 1, orchards 4 and 14) did the analyses for diseased

trees at distance L from central trees, along rows and along arrays, show that such a grouping had possibly occurred. Figures for distances L and 1.4 L, in those two orchards, are apt to contain the effect of that kind of grouping.

Another cause of grouping would be the replacement of groups of psorosis-free trees by diseased trees. One possibility in this connection is the replacement of whole groups of trees that have died or declined as a result of waterlogging, or as a result of diseases to which waterlogging is a contributing condition, in parts of the orchard. Such a grouping, however, probably would be more plainly apparent on the maps than has been the case in the orchards used in this study.

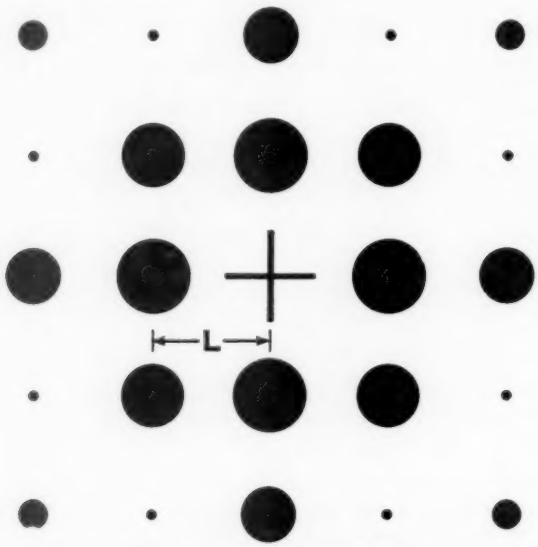

FIG. 3. Diagram illustrating the gradient of infectivity around a diseased tree. The diameters of the circles representing the 16 trees at distances L, 1.4 L, 2 L, and 2.8 L are proportional to the means of the differences in tables 1 and 2. The 8 trees represented as dots were not included in the study. These trees and others at greater distances, not shown in the diagram, would probably have shown small differences.

While the possibility of other causes of grouping cannot be dismissed, it does not seem probable that they would have acted in all the orchards used in this study, and it is safe to conclude that it has been demonstrated that a considerable percentage of trees, originally healthy in many orchards, may have contracted the disease from near-by trees that contained the virus at the time they were set out in the orchard.

There are several possible means of transmission that might be involved in the grouping of diseased trees revealed in this study; these include root-graft transmission or transmission by insects, rodents, or other animals, from tree to tree. The comparatively rapid decrease of the differences as the distance increases seems to favor transmission by root grafting. This decrease is actually a measure of a gradient of infectivity around diseased

trees. In the measuring of this gradient, all trees used as central trees are concerned; it is, therefore, the measure of a mean gradient of infectivity different from the gradient of infectivity often considered in problems of transmission of plant diseases in the field. Figure 3 represents this gradient

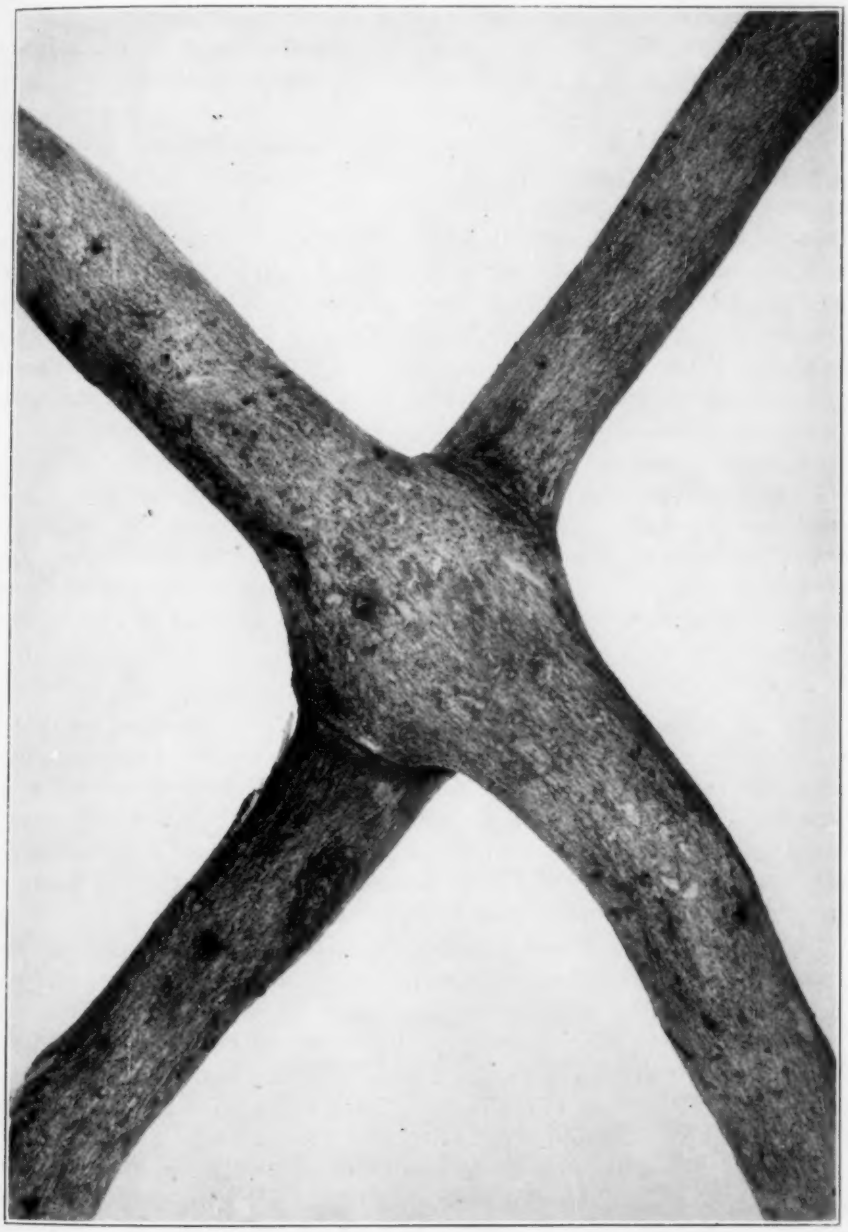

FIG. 4. Roots from adjoining trees, showing natural grafting where they had crossed. Such grafts have proved sufficient to transmit psorosis from one tree to another.

of infectivity diagrammatically. Each tree, except the central tree, is represented by a black circle, the diameter of which is proportional to the mean difference, at the given distance, for all the orchards showing a difference in favor of diseased central trees. This diagram shows that trees other than those considered in this study might have revealed transmission from the central tree if the method had been applied to them.

Observations have been made pointing to transmission by natural root grafting between roots of diseased and healthy trees. A number of natural root grafts have been found (Fig. 4) between adjacent trees in commercial orchards and between trees in the nursery. Root grafting has been shown to be a means of transmission by grafting experiments in which roots of diseased trees were grafted to healthy trees in the nursery and in the orchard. It is easily understood how a rather considerable number of new cases of psorosis may arise when the growth of the root system has reached the stage where natural root grafting is possible between adjacent trees. A number of healthy trees can be infected almost simultaneously if they are already connected by their root systems at the time one of them is naturally root-grafted to a diseased tree. Such a mode of transmission is comparable to the condition established in the preparation of map Ia.

Negative results only have been obtained in the limited number of experiments attempting transmission of psorosis by means of certain insects, and observational evidence appears to point to its not being an effective means in California. This means, however, cannot be entirely overlooked. Observational evidence, so far, suggests only a very slow and infrequent transmission from tree to tree by some such means, in addition to natural root grafting.

SUMMARY

On the maps of 14 sweet-orange orchards in California, on which psorosis-diseased trees, ranging from 3.8 to 70.6 per cent, had been recorded, counts of diseased trees around diseased trees and around healthy trees were made. The counts were made separately, first for the 4 trees closest to the diseased or healthy central trees (those at distance L from central trees), in all 14 orchards, and then, successively, for the 4 trees at distances 1.4 L, 2 L, and 2.8 L from central trees, in 11 of the orchards.

In one orchard there was a greater (nonsignificant) number of diseased trees at distance L from healthy trees than at distance L from diseased trees. In 13 orchards there were greater numbers of diseased trees at distance L from diseased trees than at the same distance from healthy trees, and the differences were significant or highly significant statistically in 11 of these 13 orchards. For these 11 orchards, differences in favor of diseased trees were found in 10, 10, and 9 orchards, at distances 1.4 L, 2 L, and 2.8 L, respectively, the differences being significant or highly significant in 7, 6, and 2 of the orchards for the respective distances. A marked decrease of the difference was observed as the distance from the central tree increased, the mean for all the differences available being 0.305 ± 0.022 for distance L,

0.262 ± 0.025 for distance 1.4 L, 0.233 ± 0.027 for distance 2 L, and 0.119 ± 0.027 for distance 2.8 L.

A separate analysis for the two trees at distance L from diseased trees and from healthy trees, along rows and along arrays, showed that in only two orchards were there significant differences in the mean number of diseased trees at distance L from diseased trees along rows and along arrays. This difference is interpreted as indicating that in those two orchards runs of diseased trees from the same budstick from diseased parent trees were set along rows at the time the orchards were planted.

Comparison with maps of fictitious orchards, on which given percentages of diseased trees were randomly distributed at given distances from randomly distributed diseased trees, showed that the differences found for the actual orchards were in some orchards comparable to those that would occur if from 2.2 to 17 per cent of the diseased trees in the orchard had become diseased as a result of transmission from diseased trees at distance L, with correspondingly decreasing percentages as the distance increased.

Among the possible causes of transmission of psorosis, root grafts occurring naturally in the orchard are considered as most likely to result in (1) the high percentage of transmission that would produce differences as great as those observed and in (2) the comparatively rapid decrease in the differences as the distance from a central tree increases. The possibility of additional means of transmission, as by insect vectors, cannot be entirely dismissed.

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CONTROL OF THE TRUFFLE IN BEDS OF THE CULTIVATED MUSHROOM

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(Accepted for publication December 30, 1943)

The invasion of mushroom beds by the weed fungus, *Pseudobalsamia microspora* Diehl and Lambert, causes a substantial annual loss. The widespread fear of this organism among mushroom growers is principally due to their lack of knowledge concerning its source and control.

The truffle has been scientifically recognized since about 1927 (2). At the present time the organism is extremely widespread and probably exists, at least to some extent, wherever mushrooms are grown.

GENERAL DESCRIPTION OF THE INFESTATION

The fungus first manifests itself at the time of the first "break," or, later, as small wefts of white to cream-colored mycelium under the sideboards. About a week later the wefts become organized into the characteristic wrinkled, flattened "calves brains," or ascocarps (Fig. 1, C). At this stage ascocarps may begin to form over the surface of the soil and can be generally found in the compost directly underneath. The infestation may be restricted to several small plots in the bed or may affect the entire bed. Frequently, only a few beds in an entire house may show signs of it. Under some conditions only the top few inches of spawned compost may be invaded, although in other cases the invasion may be more general. The mushroom mycelium disappears in infested areas; the compost becomes black and damp, and mushroom production stops. A characteristic chlorine-like odor pervades badly infested houses.

The internally borne spores are formed shortly after the ascocarps appear and are liberated when the cream-white fruiting bodies become reddish-brown and punky, with subsequent disorganization. Ascocarps on the surface begin to break down after about 6 weeks, while those in the compost remain intact for a considerably longer time. Diehl and Lambert have thoroughly described the organism (2).

GERMINATION OF THE SPORES

Truffle spores may be germinated readily by inoculating a suspension into an ordinary bottle of pure-culture manure spawn. If desired the manure spawn may be autoclaved before inoculation with truffle spores, although there is no advantage in this. Wetting the spawn is unnecessary and may even lead to failure if done to excess.

The first evidence of growth is a luxuriant and conspicuous white mycelium around the top of the bottle (Fig. 1, A). Ascocarps form abundantly between the spawn and the glass walls of the bottle. Attempts to germinate spores on other media have failed. If sterile compost is simul-

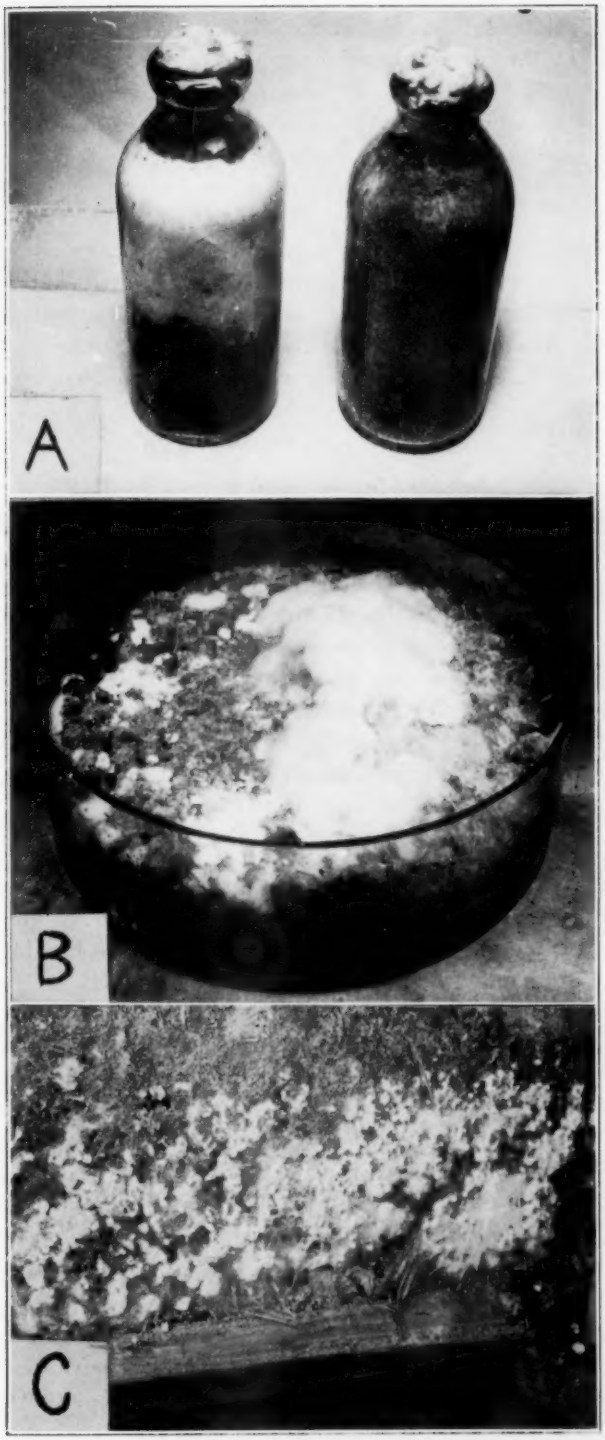

FIG. 1. Different aspects of the truffle fungus (*Pseudobalsamia microspora*) in culture. A. Mycelial stage. B. Thirty-day-old culture incubated at 74° F. C. Characteristic wrinkled ascocarp stage.

taneously inoculated with mushroom mycelium and truffle spores, the spawn will develop in the usual manner, but the subsequent appearance of the truffle is not at all conspicuous, and ascocarp production is reduced.

The optimum temperature for germination is 87° F. At this temperature growth becomes evident in about 11 days. At 60° F. the spores fail to germinate. At 72° F. an approximate incubation period of 25 days is required.

Germination in manure spawn occurs in the moisture range of 100–250 per cent on a dry weight basis. Spore germination shows a much more distinct preference for dry conditions than is commonly supposed. The optimum moisture condition for germination is about 130 per cent. Germination is retarded or may fail altogether when the moisture content exceeds 200 per cent.

FACTORS AFFECTING GROWTH

The optimum temperature for mycelial growth is 83° F. with a minimum at 52° F. and a maximum at 97° F. (1). The pH range in which growth is tolerated is 4.5–7.5, with an optimum at 6.8 (1).

In the temperature range 55–85° F., the truffle mycelium grows much more rapidly than that of the mushroom. When a Petri dish is simultaneously inoculated with both organisms and incubated within this temperature range, the mycelium of the truffle will virtually surround the mushroom mycelium in a short while.

Truffle mycelium grows in pure culture spawn in the moisture range of 100–300 per cent with optimum growth at 185 per cent on a dry weight basis.

CULTURE MEDIA

Pure-culture manure spawn is the most satisfactory medium for growth and ascocarp production. When pure-culture spawn is inoculated with truffle mycelium an abundance of fruiting bodies can be had in 9 or 10 days at 83° F. Potato-dextrose agar supports growth fairly well with limited production of ascocarps. The organism grows very weakly in sterile composted manure at pH 7 and not at all in nonsterile compost at the same pH. Growth on the former medium is considerably improved by the addition of a spawn extract. Growth is poor in ordinary garden soil, but, again, is rather good in soil taken from a bed that is producing mushrooms. It seems evident that mushroom mycelium releases a substance that is specifically stimulating to the growth of truffle. Good evidence that the truffle is not parasitic on mushroom mycelium is afforded by the fact that it will not grow in a rye spawn medium, even though mushroom mycelium flourishes on rye grains.

A rather good growth of truffle can be secured on oat hulls supplemented with garden soil. It grows well—even fruits—on moist mushroom-house boards, a factor to be taken into consideration when disinfection of the boards is contemplated.

SOURCE OF THE SPORES

At an early stage of his investigations, Lambert (4) suggested that the truffle probably inhabited the soil. Glasscock and Ware in England have brought forth evidence to support this view (3). Their observations on normal and infested beds cased with soils from different areas indicated that a particular soil source was carrying the spores. This presumptive evidence was confirmed when they succeeded in securing a growth of truffle by placing a layer of suspected soil over pure-culture spawn in a test tube and incubating at 83° F.

The following account implements the belief that the soil is the primary source of spores. A mushroom grower in Delaware converted a small stone structure near his main plant into a mushroom house. The manure, soil, and spawn used in this house and in the main plant were similar; nevertheless, every bed in the stone house was invaded by truffle, while the main plant was entirely free of the fungus. Further inquiry revealed that the manure for the small plant had been composted on a sodded area next to the regular composting ground. On Dec. 18, 1941, a number of soil samples were removed from the sodded area and placed over a 3-inch layer of manure spawn in preparation dishes 10 inches in diameter. The same procedure was followed with soil from the regular composting ground, as well as with garden soil used as a control. All dishes were incubated at 74° F. One month later the dishes containing soil from the sodded area showed a conspicuous growth of truffle (Fig. 1, B). The control and the soil from the regular composting ground were free of the fungus.

There can scarcely be any doubt that the sod was contaminated with truffle spores that were worked into the manure during the turnings of the heap. At least the upper inch of sod had been worked into the manure by this means. Later attempts to find ascocarps in the sodded area during the summer were unsuccessful. Incidentally, mushroom houses are as yet the only known habitat of the fungus.

The experience of another grower in the Oxford area is illustrative. Shortly after casing, the mycelium of the mushroom came up through the soil and formed a thin mycelial mat in several places throughout the house. Since the grower had none of the soil left with which he had originally cased his beds, he secured some nearby soil and distributed it lightly over the areas containing mycelial mats. Truffle later appeared in almost every one of these spots, whereas the rest of the house was not invaded. The house temperature was unusually high during this period and contributed to the severity of the outbreak. Again it seems very likely that the soil placed over the spots containing mycelial mats was contaminated.

Manure spawn often has been imputed as a possible carrier of spores. Distasteful as the idea is to manure-spawn makers, the possibility must be admitted. Unless the sterilization procedure is thorough, the heat-resistant spores might survive and be carried over into the beds when the spawn is planted. In bottles of compost contaminated with truffle spores before their

inoculation with mushroom mycelium, the appearance of truffle growth is not at all conspicuous, being limited to scanty ascocarp production with little evident mycelium. Since manure-spawn bottles are sold or put in storage after about 5 weeks of incubation, a small growth of this sort might go undetected.

In one particular instance a grower planted the two halves of his house with spawn from two different manufacturers. A large amount of truffle appeared in one half of the house but not at all in the other. All other factors in this house were identical. It would be difficult to avoid the conclusion that the spawn was contaminated.

The rapid spread of truffle in the mushroom industry after its recognition in 1927 might have been due to contaminated manure spawn. It has been the practice of certain spawn makers in the past to add soil to their compost; and the initial contamination of the raw material for spawn making might have occurred in this fashion. It should be emphasized, however, that the present spawn makers are keenly aware of the danger of contamination and have taken extraordinary steps to obviate it. There is very little likelihood that spawn purchased nowadays can contain living truffle spores. Since the truffle does not grow in rye spawn and there is little likelihood of rye grains being naturally contaminated, the possibility of spreading truffle spores through this medium must be discounted.

INFECTIVITY EXPERIMENTS

Beach (1) secured only occasional success when attempting to establish an infestation by inoculating the manure with spores.

In the winter of 1941 the writer made, without success, 16 separate attempts to infest mushroom beds. The spores were distributed in the compost before and after composting, after spawning, and also were applied to the casing soil. The inoculations were made in different houses in which the temperature and other conditions were regulated in a manner optimum for mushroom production. In none of these cases was the temperature favorable for the development of truffle.

On 5 occasions ascocarps that had appeared in a bed were deliberately removed and mixed with spawn of the same and different houses without resulting infection.

In another series of experiments small cans 9″ in diameter and about a foot high were filled with composted manure and placed in mushroom houses. These cans were inoculated with spores before and after the "sweat-out" and after casing. After the spawn had "run" in each can, 5 of these were removed to a room thermostatically controlled at 74° F. while the remaining ones were left in the mushroom house at about 62° F. After 6 weeks, 2 of the cans at 74° F. showed signs of truffle; neither the remaining cans at 74° F. nor those left in the house developed truffle within the next 2 months. Parts of this experiment were repeated with the invariable result that truffle did not always appear, even when the temperature was favorable.

In general, temperature appears to be a critical factor in the appearance of the weed fungus in contaminated beds; nevertheless, since growth does not always occur under favorable temperature conditions, other factors are obviously involved. This is a particularly complicating feature of the truffle problem. Further work on the factors influencing spore germination certainly is indicated. It should be pointed out that truffle spores always germinate in pure-culture manure spawn under favorable temperature conditions; hence, the answer to this problem lies in investigating the manure as it is prepared for mushroom growing.

On many occasions samples of spawned compost were taken out of mushroom houses and inoculated with truffle mycelium. In almost every case the mycelium was able to become established and form ascocarps. This was not the case when the same spawn samples were inoculated with spores. The failure to secure an infestation under apparently favorable conditions is not due to conditions adverse to mycelial development, but to conditions that suppress spore germination.

THERMAL DEATH POINT OF TRUFFLE SPORES

Spores have been reported (4) able to withstand a temperature of 180° F. for 5 hours. A series of bottles containing pure-culture spawn were inoculated with spores and subjected to various temperatures. The ability of spores to survive 180° F. for 5 hours was evident; however, they were killed after 7 hours at this temperature. Spores survived 3 hours at 200° F. In each case of survival at high temperatures there was no marked reduction of the incubation time. The spores are killed by the regular autoclaving procedure of 20 minutes at 250° F. The extraordinary resistance of the spores precludes any possible control by the use of heat.

FUNGICIDES

Stoller (5) exposed spores for 24–48-hour periods to 19 fumigants. Only ammonia, dimethylamine, quinoline, and methanol retarded germination for 4 months or longer, while the remainder were ineffective. Complete details of his tests were not given. The commonly used formaldehyde and sulphur dioxide gas were without effect. Apparently no fumigant gave a complete kill. Stoller suggests that the ammonia evolved during composting may serve to retard spore germination.

Fungicidal tests were made as follows: In the case of fumigants, ascocarps were crushed and laid upon glass slides to dry. The slide was then placed in a chamber saturated with each fumigant for the desired period of time. The spores were then washed off the slide and inoculated into duplicate bottles of pure-culture manure spawn. In the case of fungicidal liquids, the spores were suspended in water and 3 ml. of the suspension added to an equal volume of the liquid. After the desired exposure, the spores were filtered off under gentle suction on a thick asbestos pad. The pad was washed and the spores mixed into 50 ml. of water. Twenty-five ml.

of this asbestos suspension containing spores was put into each of 2 bottles of pure-culture manure spawn.

The spores were exposed for periods ranging from 1 to 6 hours. Various concentrations of liquid materials were employed. In no case were the spores killed. The following concentrations of various materials failed to kill after 6 hours: 1 per cent mercuric chloride, 2 per cent phenol, 2 per cent copper sulphate, 2 per cent ammonium hydroxide, 1 per cent glacial acetic acid, 1 per cent acetone, 1 per cent methyl alcohol, 0.50 per cent sodium ortho-di-nitro-cresolate, 0.50 per cent sodium tetra-chlorophenolate, 0.50 per cent sodium tri-chlor-phenolate. The following fumigants also were ineffective after 6 hours: formaldehyde, chlorine, sulphur dioxide, and chloropicrin. Although there was evidence of inhibition of germination in the cases of formaldehyde and ammonium hydroxide, a vigorous mycelium nevertheless appeared in the spawn bottles after 7 weeks.

CONTROL MEASURES

The soil is the primary source of spores: there are, however, various means by which the spores are actually introduced.

1. Infested soil may be added to the manure before or during composting. Many growers add soil because of the physical benefits conferred on the manure. Recently, it has become the practice to add 25–30 pounds of gypsum per ton of manure instead of soil, and this should be encouraged. It is not possible, apparently, to eradicate the spores in the soil by the use of steam or the usual fumigants.

2. The composting ground may be contaminated and spores may be worked into the manure during the turning of the heap. Composting over fresh sod or other plant-supporting ground should be avoided. Only a well-packed and well-drained composting ground should be used.

3. Spores may be brought in with contaminated casing soil. As mentioned above the ordinary techniques of soil sterilization are not effective against truffle spores. Under ordinary circumstances spores brought in with the casing soil are not likely to do any damage because the temperature usually is lowered sufficiently at this stage to prevent germination. Of course, this would not be true in unusually warm weather. In addition, soil-borne spores may serve to reinfest the following crop.

The failure of truffle spores to germinate at a temperature of 60° F. offers a positive means of controlling this weed fungus. At this temperature a perfectly normal, though slower, set of spawn can be secured. The loss in time is more than offset by lack of damage. An instance may be reported of a grower who suffered constantly from truffle until he was induced to run his spawn at a lower temperature, after which he became immediately free of it. Apparently, the question of reinfestation must be attacked in the same manner. Fungicides appear to be ineffective in eradicating the spores. Temperature control is, however, perfectly reliable and easy.

Experiment has shown that, even though truffle mycelium does grow on bed boards, the mycelium is easily killed by drying. There is probably very little trouble from this source.

Although the belief is widespread among mushroom growers that the truffle spreads from its primary site to other sites in the same or other beds, there is actually no evidence of this. Frequently only one or two spots of truffle appear in an entire house. The organism does not migrate through the compost continuously from a point of infection. Usually, it forms irregularly round patches and then stops. The universal appearance of the weed in the bed means that the spores were widespread initially.

As for possible bed-to-bed spread by means of spores, it should be pointed out that the temperature ordinarily is too low to permit their germination. Numerous experiments have confirmed this.

Growers commonly apply salt, formaldehyde, and other fungicidal materials to areas where ascocarps have appeared. This practice is not justified. In the first place the spores are resistant to the usual fungicides, and, even though they were not, it would be difficult to get at the internally borne spores, especially those contained in ascocarps deep in the manure. Secondly, by the time the application is made the infestation is already firmly established. Since secondary infection does not occur, the use of chemicals seems superfluous.

Truffle-infested areas frequently can be brought back into production if the area is not drenched with chemicals inimical to the mushroom spawn. Frequently, the invasion of the bed is superficial, involving only the top few inches of manure. In such cases there is usually healthy spawn underneath the infested area. Observation seems to indicate that the invasion tends to be superficial when the compost is packed rather tight in the bed. In such cases, the infested area can be dried up until the ascocarps have begun to desiccate and the truffle mycelium has passed its climax and disappeared. After this, water may be applied to encourage the spawn underlying the infested area to reinvade the compost and form fruiting bodies. The bed should be allowed to dry for 3 weeks or longer before it is watered again. One grower who has benefited greatly by this procedure estimates that he has been able to save about 50 per cent of his crop in infested areas. Of course when the invasion is deep and no healthy spawn remains in the bed it is futile to follow this procedure. The presence of spawn can be easily determined by inspection. The use of chemicals is not justified, even in the case of deep invasion.

The practice followed by some growers of digging a deep trench around infested areas also is not indicated, in view of the facts. It will, however, require considerable education to convince growers that the truffle does not spread from one place to another.

In removing an infested crop the grower should take particular care to dispose of the manure at a considerable distance from mushroom houses. It would be most desirable to be able to recommend a fungicide that could be

effectively used after removing an infested crop; unfortunately, no such material is known at this time. Consequently, the carry-over of spores from one crop to another is a most serious factor in the incidence of infestation. Again, temperature control must be relied on. Growers without refrigeration should by all means avoid late-spring or early-fall crops.

SUMMARY

Truffle spores were regularly germinated and ascocarps obtained by inoculating bottles of pure-culture manure spawn and incubating at 72°–85° F. Factors affecting spore germination and growth are described. Since truffle spores do not germinate at 60° F., a positive means of control is afforded through regulation of the temperature. Additional evidence is cited to show that the soil is the source of spores. In view of the fact that secondary infection does not occur, the practice of applying fungicides is not justified. When invasion of the bed is only superficial, production may be restored by drying up the infested area, followed by watering in the usual manner. A number of fungicides were found to be ineffective against truffle spores after six hours' exposure.

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FUSARIUM DISEASES OF BROAD BEAN. I. A WILT OF BROAD BEAN CAUSED BY FUSARIUM AVENACEUM VAR. FABAE N. VAR.[1]

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(Accepted for publication January 4, 1944)

INTRODUCTION

The Fusarium wilt and foot rot of broad bean caused by *Fusarium avenaceum* (Fr.) Sacc. is the most destructive disease of this crop in Yunnan, China. During the past 4 years, considerable study has been devoted to the pathogenicity of this fungus; and, as was revealed, it comprises a number of physiological strains differing from one another in ability to induce either wilt or foot rot. In the inoculation experiments conducted during the past 3 years, the typical wilt-producing strain caused the characteristic wilt, and seldom, if ever, foot rot. As far as the writer is aware this is the first record of a plant wilt induced by *Fusarium avenaceum*, and, for the sake of clearness, it is assumed that we are concerned with a new variety of the species. This paper deals with the detailed description of this disease, with its distribution in so far as it is known, with experimental inoculations, and with description and identification of the fungus.

REVIEW OF LITERATURE

Four species of *Fusarium, i.e., F. gramineum, F. vasinfectum, F. avenaceum*, and *F. orthoceras* var. *pisi*, have been reported pathogenic to broad bean by various investigators. The first record of the Fusarium root rot of broad bean was provided by Kirchner (2) in 1890. Later, a wilt of broad bean, characterized by the slender shoots and few pods, was reported by Miyake (6) from Japan in 1924. Both Kirchner and Miyake found *Gibberella saubinettii* (*F. gramineum*) to be the causal agent.

In 1920, Pape (7) reported *Fusarium avenaceum* (*F. herbarum* var. *tuberculariodes*) as causing a foot rot of broad bean. Wollenweber and Reinking (9) mentioned *F. avenaceum* f. 1 on the same host. Marchal and Verplanke (5) reported a wilt of broad bean in Belgium in 1926, and attributed the cause to *F. vasinfectum*. Linford (3), in connection with his investigation on the Fusarium wilts of peas in Wisconsin, found that *F. orthoceras* var. *pisi* is only weakly pathogenic to broad bean. Kadow and Jones (1) reported the occurrence of the pea-wilt organism on *Vicia gigantea* and Sutton's New Giant broad bean in Washington.

DISTRIBUTION OF THE DISEASE

The Fusarium wilt has been found well distributed over the important

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broad-bean growing sections of Yunnan. The first finding of the disease was at I-liang, in December, 1938. Subsequent examinations of plants from this same locality have shown it to be abundant. The gradually accumulating evidence from survey records shows it to be abundantly present in practically every section in this part of China. Many fields appeared to be completely infected.

ISOLATION OF THE FUNGUS

The basal stem portions of diseased plants were washed thoroughly in sterile water, then soaked in mercuric chloride solution (1:1000) for 20 minutes, and placed in Petri dishes in hard agar. Pure cultures were then made. The fungus may be obtained from the stem several inches above the soil surface.

SYMPTOMS OF THE DISEASE

The first symptom of the disease appears when the plants are near or at the blossoming stage. The leaves are first pale-green then light-yellow with black lesions on the leaf margin, especially on the top of the leaf. The upper leaves may be in an erect position, and may become more rigid than the normal ones. Sometimes the entire plant becomes yellow and the leaves wither progressively upwards to the terminal buds. The leaves also may become yellowish-green and quickly dried out. They are twisted, curly, and papery in texture; meanwhile, the stems turn black and wither. A plant may wilt within a week after the first symptom appears. In general, it takes 20 to 30 days to wilt completely under field conditions. Stunting of plants may be common with wilted plants.

Upon pulling any of the plants in the advanced stage of the disease it is found that they come out of the ground very easily, due to the fact that the root systems have been practically destroyed. Nearly all the fibrous roots are rotted off and the main root itself undergoes, in most cases, a dry decay. Vascular discoloration develops in the upper part of the tap-root and may extend into the stem to a considerable distance.

INOCULATION EXPERIMENTS

The ability of the fungus to infect the broad bean and produce the wilt typical of those plants from which the fungus has been isolated many times, has been established through inoculation experiments both in the field and under laboratory conditions. Infection takes place when a spore suspension of the fungus is added to the soil when the plants are several inches high.

On January 3, 1939, inoculations were made as follows: Twenty 60-inch-high broad-bean seedlings were grown in sterilized soil in pots with one plant per pot and inoculated by pouring a spore suspension on the basal stems. When examination was made on April 17, 1939, at which time the experiment was discontinued, wilt was found on 8 of the 20 plants. Check plants remained healthy. The fungus was isolated from these 8 plants and its identity determined.

On January 14, 1939, broad bean seeds were soaked for 30 minutes in mercuric chloride solution (1:1000), washed in sterile water, and planted in 12-inch pots containing sterilized soil at the rate of 5 seeds per pot. After these plants attained a height of 12 inches, 40 of them were inoculated with the fungus by pouring the spore suspension just on the base of the stems. Readings were made on April 29, 1939. Twenty-one of the 60 plants wilted. No foot rot was observed. The wilted plants were examined and the fungus was found growing on the roots causing browning discoloration of the vascular bundles on the tap roots.

In the fall of 1939, infection was obtained by inoculating seedlings of 3 varieties of broad bean. They were grown in 12-inch pots containing sterilized pure sand. The seeds were sown on October 19, 1939. The seedlings were inoculated 4 times at an interval of 20 days from November 20 of that year until January 19, 1940. The inocula were taken from cultures on steamed broad-bean stems. Heavy spore suspension was prepared. Infections were obtained in the months of March and April on all varieties and the checks were found to be sound. The percentages of infection were 38.2, 62.7 and 51.1, respectively.

Several other experiments similar to the above have been conducted at various times and have always yielded a high or low percentage of infection.

No evidence has been obtained that *Fusarium avenaceum* var. *fabae* is a cause of wilts on common bean, cowpea, tomato, wheat, oats, corn, carrot and pea.

DESCRIPTION AND IDENTIFICATION OF THE CAUSAL FUNGUS

Morphology. The following media were employed in the study and identification of the causal fungus: steamed rice, broad-bean stem and pod, locust stem and root, Sophora stem and root, Irish-potato plug, hard oat agar, hard potato-dextrose (2 per cent) agar, corn-meal agar, broad-bean-decoction agar (100 g. of seed per 1000 cc. distilled water), Czapek's agar, and 1 and 2 per cent dextrose standard nutrient agars. The agar media were in both test tubes and plates. The media were prepared according to the recommendations of Sherbakoff (8) and the committee on taxonomy of Fusarium (10).

In determining the size of macroconidia, at least 100 and sometimes 200 or more spores were measured. The measurements of them in various media are as follows:

Broad-bean stem; cultures 38 days old; macroconidia from sporodochia:
Macroconidia—
4-septate, 1 per cent, 45.6×4.4 μ.
5-septate, 99 per cent, 51.3×4.2 (45.3–66.1 × 3.5–4.7) μ.

Broad-bean stem; culture 40 days old; macroconidia from sporodochia:
Macroconidia—
3-septate, 1 per cent, 34.8×3.5 μ.
4-septate, 13 per cent, 41.6×3.9 (38.4–48.7 × 3.5–3.8) μ.
5-septate, 86 per cent, 46.3×3.5 (38.3–55.7 × 3.5–4.2) μ.

Broad-bean-decoction agar; cultures 27 days old; macroconidia from sporodochia:
Macroconidia—
4-septate, 2 per cent, 56.8×3.8 ($53.9–59.3 \times 3.7–3.8$) μ.
5-septate, 97 per cent, 58.6×3.7 ($50.4–73.1 \times 3.5–5.2$) μ.
6-septate, 1 per cent, 73.1×4.5 μ.

Broad-bean-decoction agar plate; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 5 per cent, 50.8×3.7 ($45.2–55.7 \times 3.5–3.8$) μ.
5-septate, 94 per cent, 57.0×3.7 ($47.0–67.9 \times 3.5–4.0$) μ.
6-septate, 1 per cent, 62.4×3.8 μ.

Broad-bean-decoction agar plate; culture 40 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 95 per cent, 56.3×3.8 ($43.5–67.9 \times 3.5–4.4$) μ.
6-septate, 5 per cent, 61.9×4.0 ($53.9–67.9 \times 3.7–4.5$) μ.

Broad-bean-decoction agar plate; culture 44 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 98 per cent, 55.5×3.6 ($48.7–67.9 \times 3.5–3.7$) μ.
6-septate, 2 per cent, 61.2×3.6 ($60.9–62.6 \times 3.5–3.7$) μ.

Pea pod; culture 25 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 5 per cent, 44.2×3.7 ($40.0–48.9 \times 3.5–4.0$) μ.
4-septate, 31 per cent, 47.7×3.5 ($40.0–62.6 \times 3.5–3.8$) μ.
5-septate, 63 per cent, 53.9×3.5 ($45.2–73.1 \times 3.5–3.8$) μ.
6-septate, 1 per cent, 66.1×3.8 μ.

Pea stem; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 4 per cent, 47.6×3.8 ($45.2–55.7 \times 3.7–4.0$) μ.
5-septate, 94 per cent, 57.4×3.8 ($43.5–67.9 \times 3.5–4.0$) μ.
6-septate, 2 per cent, 63.0×3.8 ($64.4–69.6 \times 3.7–4.0$) μ.

Pea stem; culture 38 days old, macroconidia from sporodochia:
Macroconidia—
5-septate, 95 per cent, 56.2×4.4 ($43.5–71.7 \times 3.7–4.5$) μ.
6-septate, 5 per cent, 63.3×4.0 ($64.4–76.6 \times 3.8–4.4$) μ.

Pea stem; culture 40 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 2 per cent, 46.1×3.5 ($45.2–47.0 \times 3.5–3.7$) μ.
4-septate, 7 per cent, 48.7×3.5 ($41.7–55.7 \times 3.5–3.7$) μ.
5-septate, 90 per cent, 52.2×3.5 ($43.5–67.9 \times 3.5–3.7$) μ.
6-septate, 1 per cent, 73.1×3.5 μ.

Sophora stem; culture 38 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 8 per cent, 48.7×3.7 ($43.5–55.7 \times 3.7–3.8$) μ.
5-septate, 90 per cent, 51.9×3.7 ($45.2–59.2 \times 3.7–3.8$) μ.
6-septate, 2 per cent, 73.1×3.8 μ.

Locust stem; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 1 per cent, 51.5×3.7 μ.
5-septate, 91 per cent, 61.8×3.6 ($48.7–73.1 \times 3.5–4.0$) μ.
6-septate, 8 per cent, 70.3×3.7 ($62.6–74.8 \times 3.5–4.0$) μ.

Locust root; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 4 per cent, 53.6×3.6 ($50.1–59.2 \times 3.5–3.8$) μ.
5-septate, 96 per cent, 57.4×3.6 ($48.7–66.1 \times 3.5–3.8$) μ.

Locust root; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 10 per cent, 47.9×3.6 ($38.8–52.2 \times 3.5–3.8$) μ.
4-septate, 31 per cent, 50.8×3.6 ($40.0–55.7 \times 3.5–3.7$) μ.
5-septate, 59 per cent, 56.0×3.7 ($48.7–62.6 \times 3.5–3.8$) μ.

Locust root; culture 45 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 5 per cent, 53.6×3.7 ($48.7–60.9 \times 3.5–3.7$) μ.
5-septate, 95 per cent, 48.5×3.7 ($48.7–69.6 \times 3.5–3.8$) μ.

Potato plug; culture 44 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 3 per cent, 46.5 × 3.6 (43.5–48.7 × 3.5–3.7) μ.
5-septate, 95 per cent, 52.9 × 3.6 (43.6–64.4 × 3.5–3.8) μ.
6-septate, 2 per cent, 56.6 × 3.6 (50.5–62.6 × 3.5–3.7) μ.

Hard oat agar plate; culture 30 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 2 per cent, 42.8 × 3.5 (33.1–50.5 × 3.8–4.0) μ.
4-septate, 8 per cent, 42.1 × 3.8 (40.0–59.2 × 3.7–3.8) μ.
5-septate, 88 per cent, 54.4 × 3.8 (47.0–73.1 × 3.5–4.2) μ.
6-septate, 2 per cent, 64.0 × 4.0 (62.6–67.9 × 3.8–4.2) μ.

Hard oat agar tube; culture 43 days old, macroconidia from sporodochia:
Macroconidia—
4-septate, 3 per cent, 56.7 × 3.6 (55.7–57.4 × 3.5–3.7) μ.
5-septate, 90 per cent, 63.0 × 3.7 (52.5–71.7 × 3.5–3.7) μ.
6-septate, 6 per cent, 69.6 × 3.6 (60.9–73.1 × 3.5–3.7) μ.
7-septate, 1 per cent, 73.1 × 3.7 μ.

Czapek's agar plate; culture 27 days old, macroconidia from sporodochia:
Macroconidia—
3-septate, 7 per cent, 40.0 × 3.7 (31.3–45.2 × 3.7–3.8) μ.
4-septate, 23 per cent, 42.2 × 3.6 (38.3–52.2 × 3.5–3.8) μ.
5-septate, 70 per cent, 49.1 × 3.7 (38.3–57.4 × 3.5–3.8) μ.

Averages:
3-septate, 1.47 per cent, 42.8 × 3.6 (34.8–47.9 × 3.5–3.8) μ.
4-septate, 7.98 per cent, 49.4 × 3.7 (41.6–56.7 × 3.5–4.4) μ.
5-septate, 88.53 per cent, 55.4 × 3.8 (46.3–63.7 × 3.5–4.2) μ.
6-septate, 0.05 per cent, 73.1 × 3.7 μ.

The measurements of 200 macroconidia taken from the root of an artificially infested plant are as follows:

Macroconidia from sporodochia—
3-septate, 12.5 per cent, 39.2 × 4.4 (44.1–48.7 × 3.7–5.2) μ.
4-septate, 10 per cent, 47.7 × 4.7 (36.5–53.9 × 3.8–5.2) μ.
5-septate, 75 per cent, 56.7 × 4.7 (41.8–69.6 × 3.8–5.2) μ.
6-septate, 2 per cent, 65.7 × 4.8 (60.9–69.6 × 3.8–5.2) μ.

From the foregoing spore measurements, it is seen that the macroconidia produced in sporodochia on cultural media and on the infected plants agree fairly well as to spore size and the distribution of the spore septation, with 5-septate being the predominant. The diameters of spores from diseased plants are, however, greater than those of spores produced in culture media.

The pseudopinnotal spores, on the other hand, vary greatly in shape and size, as well as in number of septations. For example: certain measurements of macroconidia taken from pseudopinnotes are given below:

Standard nutrient agar tube (1% dextrose); culture 20 days old, macroconidia from pseudopinnote:
Macroconidia—
3-septate, 16 per cent, 31.8 × 3.8 (27.8– 34.8 × 3.7–4.2) μ.
4-septate, 4 per cent, 47.9 × 3.9 (45.2– 50.1 × 3.7–4.2) μ.
5-septate, 18 per cent, 49.1 × 4.0 (40.6– 59.2 × 3.7–4.4) μ.
6-septate, 14 per cent, 58.4 × 4.4 (48.7– 67.9 × 3.8–4.2) μ.
7-septate, 22 per cent, 67.5 × 4.1 (55.7– 76.6 × 3.7–4.2) μ.
8-septate, 10 per cent, 76.6 × 4.7 (66.1– 83.5 × 3.7–4.2) μ.
9-septate, 12 per cent, 76.6 × 4.7 (69.6– 87.0 × 3.7–4.2) μ.
10-septate, 2 per cent, 92.2 × 4.7 (83.5–100.9 × 4.2–5.2) μ.

Standard nutrient agar tube (2% dextrose); culture 20 days old, macroconidia from pseudopinnote:
Macroconidia—
0-septate, 6 per cent, 19.1 × 3.6 (18.1–20.9 × 3.5–3.7) μ.

1-septate, 28 per cent, 19.4×3.6 ($15.7–22.6 \times 3.5–4.5$) μ.
2-septate, 24 per cent, 24.1×3.7 ($19.1–31.3 \times 3.5–4.4$) μ.
3-septate, 32 per cent, 35.0×3.6 ($20.9–38.3 \times 3.8–4.2$) μ.
4-septate, 4 per cent, 40.0×3.7 ($38.3–41.7 \times 3.7–3.8$) μ.
5-septate, 6 per cent, 45.8×3.7 ($43.5–50.5 \times 3.5–3.8$) μ.

Hard oat agar plate; culture 20 days old, macroconidia from sporodochia:

Macroconidia—

1-septate, 2 per cent, 27.8×3.8 μ.
2-septate, 2 per cent, 24.4×3.8 μ.
3-septate, 8 per cent, 30.5×4.4 ($24.4–48.7 \times 3.5–5.2$) μ.
4-septate, 16 per cent, 37.2×4.2 ($31.3–48.7 \times 3.7–5.0$) μ.
5-septate, 30 per cent, 46.9×4.3 ($36.5–66.1 \times 3.7–4.8$) μ.
6-septate, 16 per cent, 57.9×3.9 ($45.2–69.6 \times 3.7–4.8$) μ.
7-septate, 18 per cent, 71.9×4.4 ($67.9–79.0 \times 3.5–5.2$) μ.
8-septate, 2 per cent, 83.2×3.9 μ.
9-septate, 2 per cent, 92.2×3.9 μ.
10-septate, 0 per cent.
11-septate, 2 per cent, 102.6×3.9 μ.
12-septate, 2 per cent, 107.4×3.9 μ.

CULTURAL CHARACTERS

Rice: Twenty-five-day-old cultures are white, pink, amber-yellow, forming a medium-dense and matted mass of mycelium over the rice (4). The mycelium may be white, pink, ivory, or lilac below. Sclerotia are produced in great abundance.

Broad-bean-decoction agar: Twenty-five-day-old cultures bear a scant, thin, loose and white mycelial growth. Rufous-orange sporodochia are formed in great abundance.

Corn-meal agar: Twenty-five-day-old cultures bear a scant, thin, white, cream, pinard-yellow or chrome-lemon-yellow aerial growth.

Potato-plug: One-month-old cultures show a compact, dense, thick or matted growth of mycelium that is white, Jasper-pink, wild-rose or ash-rose. The surface of the medium is wrinkled, and powdery in appearance. At the base and the side of the slant, the color of the mycelium is marsh-rose, pepper-red and slate.

Hard oat agar: One-month-old cultures form a dense, thick, matted, white, lilac, Tango-pink, leather to buttercup-yellow and France-rose mycelial growth. The mycelium may be powdery in places. Holly-berry-red to hyacinth-red is seen in the substratum. Mycelium attached to wall at base of slant is lilac, Grecian-rose, slate, rufous-salmon, and primrose-yellow. Sporodochia small, slimy, spherical to subspherical, solitary or aggregated, and rufous-orange. Pseudopinnotes mandarin to rufous-orange.

Czapek's agar: One-month-old cultures are characterized by dense, compact, somewhat matted, pink to raspberry-red mycelium. When spores are produced in abundance, culture is rufous-salmon, slimy, and conspicuously matted.

Standard nutrient agar (plus 1 or 2 per cent dextrose): One-month-old cultures form abundant, compact, dense, and pinkish mycelial growth with powdery mycelial masses. Chimney-red, pink, lilac and slate lines are seen at the base of the culture.

Broad-bean pod: One-month-old cultures are characterized by dense, compact, white, coral to candy-pink or raspberry-red powdery mycelial growth. Rufous-orange and slimy sporodochia of various sizes are produced.

Broad-bean stem: Young cultures are characterized by an extremely fine, fluffy, and cottony-white mycelial growth. In old cultures, there is an abundant, more or less compact, cottony, white, bluish-rose, light- to sulphine-yellow growth with small, cream-color powdery masses in places on the mycelium. Sporodochia are found over the stems, in places on the mycelium, or even on the moist cotton at the bottom of the tubes.

Pea pod: One-month-old cultures form abundant, slightly compact to long, loose cottony, matted, white, cream, pink, spruce and pyrite-yellow mycelial growth with cream-color powdery masses in places on the mycelium.

Pea stem: One-month-old cultures show thin, cottony, matted, white, blush-rose, sulphine-yellow mycelial growth with cream to light-rose powdery masses in places on mycelium. Sporodochia are produced over stem, in places on mycelium, or even on moist cotton at base of tube.

Locust stem: One-month-old growth is scant, loose, thin, cottony, white, and pink. Sporodochia are produced in places on mycelium, over twigs, or on moist cotton.

Locust root: When one-month-old, scant, white mycelial growth with cream-color powdery masses in places on the mycelium are produced.

Sophora stem: One-month-old cultures form moderate growth of loose or compact, thin, cottony, white and blush-rose mycelial growth usually powdery in appearance.

Sophora root: At first the aerial mycelium is white, very scant, but fine and cottony. In two-month-old cultures still scant, white in places, usually light-blush-red.

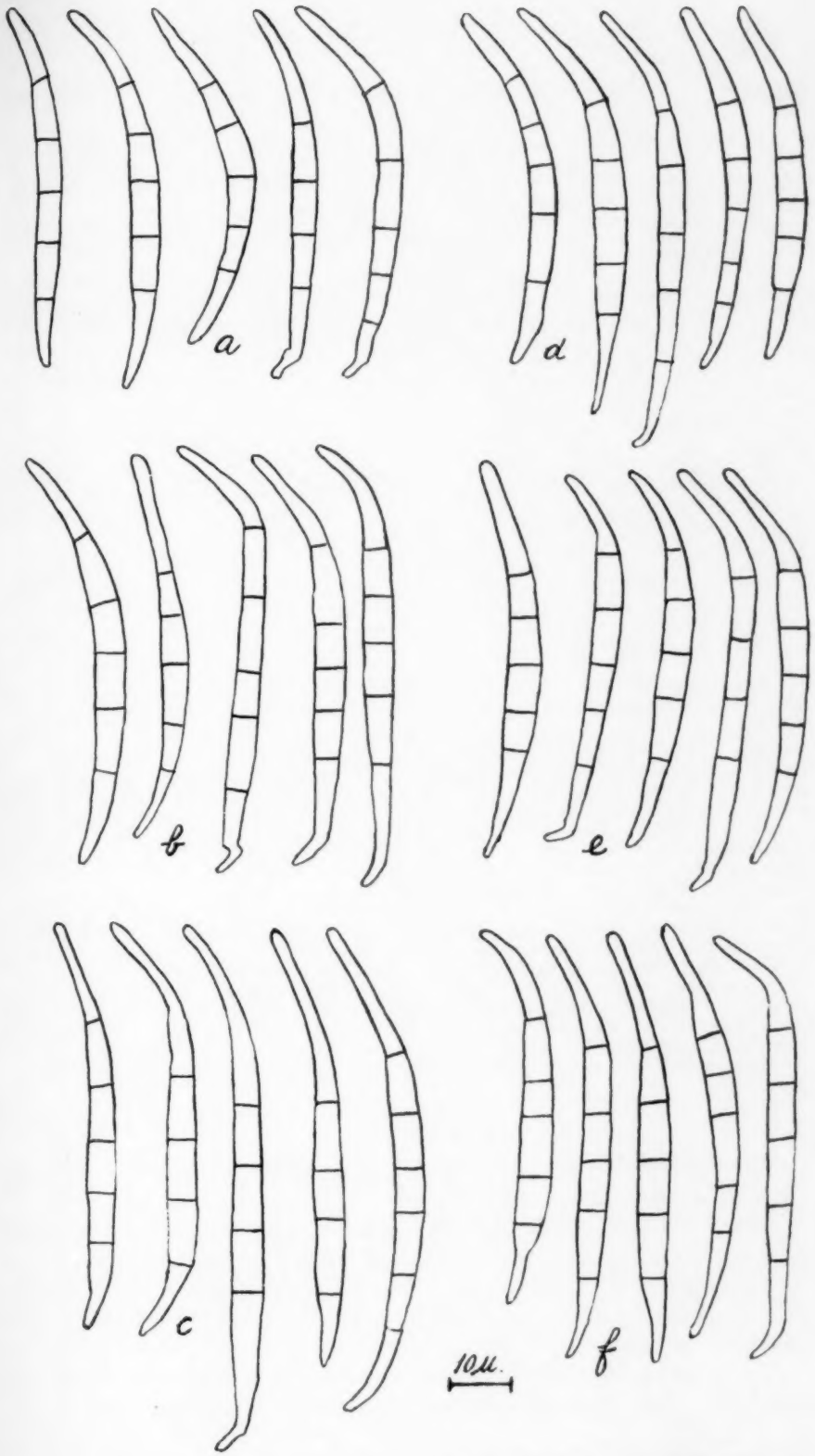

FIG. 1. Macroconidia of *Fusarium avenaceum* var. *fabae:* (a), from 37-day-old culture of steamed broad-bean stem; (b), from 30-day-old culture of broad-bean-decoction agar tube; (c), from 30-day-old culture of locust stem; (d), from 30-day-old culture of hard oat agar tube; (e), from 30-day-old culture of pea stem; and (f), from 30-day-old culture of locust stem.

Potato-dextrose (2 per cent) agar plate: One-month-old plates form a medium dense, thick, loose or matted pinkish mycelial growth over the surface. The mycelium in the substratum is Isabella, and absinth-yellow. Spots of Bokhara to cyclamen-purple with tourmaline-pink outlines are seen.

Hard oat agar plate: One-month-old cultures form matted, compact and white to blush-rose mycelial growth. In the substratum, the mycelium is raspberry to blush-rose with ruby or garnet, zonate.

Corn-meal agar plate: Mycelial growth of 1-month-old cultures, thin, loose, cottony and white to pink. The mycelium in the substratum is Isabella, absinth-yellow with few zones. Mycelial knot seen as spot of Bokhara-purple, cyclamen, with pinkish outline scattered in the plate. The knot, when examined microscopically, shows heavy and thin hyphae clamping together to form knots of various sizes.

Broad-bean agar plate: In 1-month-old culture, the aerial mycelium absent or scant. When present, only few white, thin mycelial tufts scattered in the plate. Sporodochia are, in general, produced in great abundance in this medium.

Czapek's agar plate: One-month-old cultures form thick, matted, compact and white to pinkish aerial mycelium. In the substratum, mycelium is sultan- to ruby-red, zonate.

Standard nutrient agar plate (1 or 2 per cent dextrose): One-month-old cultures have an abundant, loose or compact, matted and white to blush-rose mycelial growth. In the substratum, the mycelium is buttercup-yellow to buff and raspberry to blush-rose with crimson, mineral-red, ruby-red, cardinal-red or slate zones.

In general, sporodochia are readily produced on many kinds of media. They may be produced over the media, in places on the mycelium or even on the cotton at the bottom of the tubes. Pseudopinnotes are less frequently produced. Microconidia are only rarely seen. Chlamydospores have not been found in any of the media tested. Sclerotia are produced abundantly on steamed rice and to a lesser extent on potato plug. The stromatic structures are occasionally found.

The morphologic characters (Fig. 1) of the fungus, as determined from cultures of various media, are as follows:

Microconidia absent, or rarely produced, oval, oblong or kidney-shaped; 0–1-septate, 0-septate, 10.7×3.7 (8.7–13.9 × 3.5–3.4) μ; 1-septate, 13.9×3.4 (10.4–15.7 × 3.3–3.4) μ. Macroconidia in sporodochia, curved-spindle-shape, awl-shape, thread-like, or anguliform, long, comparatively narrow and slender, broader in middle, with long end cells, the cells detached when old, often slender, pointed and in most cases sharply bent, pedicellate or more or less 4-celled, typically 5-septate, 55.4×3.8 (46.4–63.0 × 3.5–4.2) μ, rarely 7-septate, exceptionally up to 12-septate; 3-septate, 42.8×3.6 (34.8–47.9 × 3.5–3.8) μ; 4-septate, 49.4×3.7 (41.6–56.7 × 3.5–4.4) μ; 6-septate, 62.9×3.8 (56.6–73.1 × 3.5–4.5) μ; 7-septate, 73.1×3.7 μ. Macroconidia pseudopinnate varying greatly in size, shape and number of septation; from 0–12-septate. Sporodochia spherical or irregular in outline, often fluid or slimy, shiny and rufous-orange. Pseudopinnotes less frequently produced, spreading, slimy, shiny and rufous-orange. Sclerotia, deep blue-black, rough, oval, round or irregular in size, large 1.4 mm. in diameter (0.2–2.5 mm.). Chlamydospores, absent.

From a comparison with descriptions given by Wollenweber and Reinking (9), it is evident that this fungus is in the section Roseum of the genus *Fusarium*, and within that group lies close to *Fusarium avenaceum*. It differs from *F. avenaceum*, *F. avenaceum* forma 1, *F. avenaceum* var. *pallens* and *F. avenaceum* var. *volutum* (9) in its ability to produce a typical wilt of broad-bean and is, accordingly, considered as a new variety of this species.

SUMMARY

A wilt disease of broad bean caused by *Fusarium avenaceum* var. **fabae** var. nov., is herein described.

The disease is known to occur in the important bean-growing sections of Yunnan, China, where it causes considerable damage to the crop.

The leaves of the diseased plants first turn greenish-yellow, then wither, and the plants eventually die. The vascular regions of the infected plants, especially the tap root and basal stem, are shaded brown to dark-brown.

The causal fungus of this disease is described as *Fusarium avenaceum* var. *fabae*. Its morphology and cultural characters are described.

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STUDIES ON BACTERIAL CANKER OF TOMATO

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(Accepted for publication January 3, 1944)

INTRODUCTION

Bacterial canker of tomato caused by *Phytomonas michiganensis* (E.F.S.) Bergey *et al.*, 1923, is the major bacterial disease of field-grown tomatoes in California. Although the disease is known to be seed-borne, the literature on the biology and related features of the pathogen is very limited. This paper presents some additional data on certain aspects of this disease.

VARIANTS

Pink and white strains of *Phytomonas michiganensis* have been reported (3, 4, 5). The white strain was obtained from the yellow (normal) strain and is stable under laboratory conditions, while the pink strain is unstable, yielding both white and yellow strains (5). The white strain is pathogenic to tomato, while the pink strain is only slightly pathogenic, or, in many instances, non-virulent (5). The writer has studied both the white and the pink strains of the organism and compared them with several isolations of the yellow or normal strain.

The white strain was first obtained in the laboratory as a variant of the yellow strain when the latter was grown in a beef-extract-peptone agar medium for 2 months. Pure culture studies and pathogenicity tests showed the white strain to be *Phytomonas michiganensis*. The physiologic studies were made with cultures that were passed 3 times through tomato plants. There were some differences between the white and the yellow strains when the organisms were grown in SAB synthetic medium (12) for 6 weeks. Thus, the white strain produced some gas in galactose, glycerine, levulose, and lactose in 6 weeks, while no trace of gas was observed for the yellow strain. Also, the white strain was twice isolated from field material in association with the yellow strain.

Two Strains

A pink strain of *Phytomonas michiganensis* was obtained from an infected tomato and the white-rough strain from spontaneous dissociation in a test tube. The colonies of the pink strain were small, delicate pink, glistening, and spread out with age. The slant growth was pink with a yellowish hue. The rough strain was similar to the white (smooth) strain in its behavior as to sugars in SAB synthetic media. On potato-dextrose(2%)-peptone(1%)-agar(2%) plates, colonies began to appear after 4 days' incubation at 28° C., and at first were minute, gradually becoming raised and wrinkled over the surface of the colony. En masse growth on slants is not slimy but rather dry, soon showing numerous small folds imparting a

wrinkled appearance. The growth was not stringy, but compact, becoming flocculent in saline solution (0.85% NaCl). The culture retained its characteristics on an artificial laboratory medium (beef-peptone agar) for a year. On potato plugs it gave smooth colonies after a month's growth. In pathogenicity tests, all the variants studied were pathogenic to tomato plants, but in varying degree. The white-smooth and white-rough strains were pathogenic in about the same degree and did not form open cankers on tomato stems. These two strains caused slight wilting of the leaflets, or of the whole leaf, and darkening of the cambial and vascular region. Plants inoculated with these strains grew to maturity and produced fruits. Numerous unsuccessful attempts were made to isolate the organisms of these strains from tomato fruits of plants that had previously been artificially inoculated. The pink strain is less pathogenic than the white or white-rough strain. Green tomato fruits, previously treated for 2 minutes with a 1:1000 solution of corrosive sublimate, washed in sterile distilled water, and then sprayed with the pink strain, showed minute, dark-brown to black spots that, on culturing, yielded the pink strain.

HOST RANGE

Phytomonas michiganensis is reported only on tomato, except for the statement of E. F. Smith (11) on the possible infection of potatoes. The red currant tomato, *Lycopersicum pimpinellifolium* Mill., is only slightly susceptible to the tomato canker organism, infection being slow and of a mild type, *i.e.*, cankers are not readily formed and the plant is not killed. The symptoms consist of a discoloration of the cortex and vascular region. Attempts to isolate the organism from fruits of the artificially infected plants were unsuccessful. Tree tomato (*Cyphomandra betacea* Sendt) is stunted and shows a black discoloration in the fibrovascular system after artificial inoculation with *P. michiganensis,* but never develops cankers nor does it die. *Solanum nigrum* L. var. *guineense* L., behaves similarly when inoculated.

Tobacco (*Nicotiana tabacum* L.) failed to become infected, while *N. glutinosa* L. was readily infected and the organism recovered. Yellowing and wilting of the leaves occurred and occasionally very small cankers developed on the stems.

TESTS WITH INSECTS

The insects reported below were reared in the greenhouse on healthy, muslin-covered plants most favorable for their development. Generally, 25 to 50 insects were transferred to diseased tomato plants from which transfers of 5 to 15 insects were later caged on healthy young tomato seedlings in individual pots previously kept insect-free by frequent fumigation. Infested plants were held under observation for 2 months. The disease failed to develop in any case where contaminated insects were used. Furthermore, the canker organism was not isolated from the mouth parts or the internal organs of the insects that had fed on diseased plants. The following insects were tested: green peach aphid (*Myzus persicae* Sulz.), tarnished plant bug

(*Lygus pratensis* L.), onion thrips (*Thrips tabaci* Lind.), and the twelve-spotted cucumber beetle (*Diabrotica duodecimpunctata* Fabr.).

INFECTION BY THE TOPPING KNIFE

By cutting off the tips of young tomato seedlings with a contaminated knife a higher percentage of infection results than does from the needle-prick method of inoculation.

Fifty young, thrifty tomato plants (lot 1) were inoculated by needle pricks and 50 plants (lot 2) by cutting off the tips with a sharp dissecting knife, previously contaminated with the organism from agar slants. The plants were kept under observation for 30 days. All the plants in lot 2 became infected, while only 4 per cent of those in lot 1 contracted the disease.

In another test, 30 vigorous, healthy tomato plants, consecutively numbered, were grown in individual pots. A diseased plant was cut with a cartilage knife so as to expose the entire blade to diseased tissue. The tips of the 30 plants were then cut off with the contaminated knife in a manner simulating commercial practice, resulting in 30 distinct cases of bacterial canker that clearly originated at the cut surface. Another series of 47 plants were cut with a similarly contaminated knife, and 37 developed distinct cankers originating at the cut surface.

Three flats of healthy tomato plants, containing a total of 104 plants, were cut with a knife contaminated by cutting a diseased plant. Twenty-nine out of 33 plants in flat 1, 32 out of 33 in flat 2, and 11 out of 38 in flat 3 developed the disease. This indicated that canker can be carried to numerous plants on the knife once it becomes contaminated.

Healing of the knife cut is a slow process, as shown by the following greenhouse experiments. One lot of 125 greenhouse-grown tomato plants, averaging 6 inches in height, was divided into 5 groups. The growing points of all plants were decapitated with a very sharp knife. One group of 25 plants was untreated and served as checks for the other 4 groups. All plants in the remaining group were smeared on the cut surface with a heavy paste of *Phytomonas michiganensis* at the following intervals after wounding: 24, 48, 72, and 96 hours. The final reading was made in the greenhouse after 30 days. All of the plants smeared with *P. michiganensis* 24 hours to 72, inclusive, after wounding, became infected. No disease developed on any plants smeared 96 hours after wounding.

INFECTION BY HANDLING

Fifty young plants were handled as a grower would do when pulling plants from a plant bed for transplanting. First, a diseased plant was pulled rather roughly, and then the same treatment was given to 50 consecutive plants numbered from 61 to 110. Of these, 66, 78, 80 and 83 developed canker. This demonstrated that healthy plants may be infected by the hands during the operation of transplanting.

These data indicate two ways in which the disease may be spread under commercial field conditions, and are in agreement with the observations of Müllers (9) and others.[1]

ROOT INOCULATIONS

The writer's experiments with root inoculations of tomato plants gave negative results. One hundred tomato plants, grown in individual pots, which had had their roots cut either by a contaminated knife or a clean knife followed by pouring a heavy suspension of *Phytomonas michiganensis* into the soil around the injured roots, remained healthy. This is not in accordance with the statement of Blood (2) that "wounded roots appear to be highly favorable infection courts," nor with the experiments of Orth (10).

ATOMIZER INOCULATION

Attempts to induce the disease in young tomato plants kept in moist chamber for 24 hours prior to spraying with a heavy suspension of *Phytomonas michiganensis* in distilled water were unsuccessful. Perhaps, because of the non-motility of the tomato-canker organism, the stomata are apparently unsatisfactory infection courts.

EFFECT OF GERMICIDAL TREATMENTS ON THE ORGANISM

The effect of various concentrations of alcohol, brilliant and malachite green, and rosaniline hydrochloride dyes on the organism and on tomato seed have been investigated. The dyes were dissolved in water and in alcohol of different strengths. In preliminary tests, potato-dextrose-peptone agar was prepared and enough alcohol added to make 5, 10, 15, 20, 25, 30, 35, and 40 per cent. The alcohol was added aseptically after melting the agar. The medium was then poured into Petri dishes and, after solidification, streaked with a heavy suspension of *Phytomonas michiganensis*. Control plates were without alcohol. All plates were incubated at 28° C. No growth was observed on any of the alcohol plates. Control plates developed good growth in 2 days.

Similarly, a dye agar containing 1:1000 and 1:2000 concentrations of brilliant and malachite green and rosaniline hydrochloride was prepared. The dyes and medium were sterilized simultaneously. No growth of *Phytomonas michiganensis* occurred in any of these concentrations after 10 days. Tests were then conducted to determine how long it takes each of these solutions to kill the tomato-canker organism (Table 1). Two cc. of each solution was used in agglutination tubes. A loopful of an agar-slant growth of the canker organism was introduced into a test tube, with the test solution, and vigorously shaken. Streaks were made on potato-dextrose-peptone agar plates at intervals of 5, 10, 15, and 60 minutes. All plates were incubated at 28° C. for 2 weeks and records taken once every 2 days (Table

[1] The writer is indebted to Professor J. B. Kendrick, Division of Plant Pathology, University of California, Davis, for some of the data on transmission of the disease by the contaminated knife.

TABLE 1.—*Effect of alcohol and 1 to 1000 alcoholic and water solutions of dyes on Phytomonas michiganensis in water suspension*

Treatment	5% alcohol					30% alcohol					Water (H_2O)				
	Exposure in minutes and hours														
	5	10	15	60	24 h.	5	10	15	60	24 h.	5	10	15	60	24 h.
Malachite green	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brilliant green	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0
Rosaniline hydrochloride	+	+	+	+	0	0	0	0	0	0	+	+	+	0	0
Control	+	+	+	+	+	0	0	0	0	0	+	+	+	+	+

+ = organisms grew upon streaking.
0 = no growth after streaking.

1). It is interesting to note that the addition of alcohol to the dye somewhat lowers its germicidal effect (Table 1). Thus, while a water solution (1:1000) of brilliant green and malachite green killed the organism in 5 minutes, it required 10 and 15 minutes, respectively, when 5 per cent alcohol was added.

EFFECT OF GERMICIDES ON SEED GERMINABILITY

The effect of the above-mentioned materials on tomato seed also was investigated (Table 2). The same procedure was used, except that the seeds were thoroughly washed in distilled water immediately after treatment and subsequently placed on slightly moist filter paper in Petri dishes for germination at 28° C. Tomato seed was not injured by the concentrations effective in killing the tomato-canker organism, as shown in table 2.

EFFECT OF HEAT ON GERMINABILITY OF THE SEED

The writer also studied the effect of dry heat (relatively high temperatures) on the germination of tomato seed (var. Santa Clara Canner). In experiments on the viability of tomato seeds exposed to dry heat, Miss Jozefowicz found (6, 7) that seeds withstood dry heat up to 79.5° C., even

TABLE 2.—*Effect of 1 to 1000 alcoholic and water solutions of dyes on the germination of tomato seed*

Treatment	5% alcohol					30% alcohol					Distilled water				
	Exposure in minutes and hours														
	5	10	15	60	24 h.	5	10	15	60	24 h.	5	10	15	60	24 h.
Malachite green	4+	4+	4+	3+	+	4+	4+	4+	3+	0	4+	4+	4+	4+	4+
Brilliant green	4+	4+	4+	4+	+	4+	4+	4+	2+	0	4+	4+	4+	4+	4+
Rosaniline hydrochloride	4+	4+	4+	4+	+	4+	4+	4+	3+	0	4+	4+	4+	4+	4+
Control	4+	4+	4+	4+	+	3+	3+	3+	3+	0	4+	4+	4+	4+	4+

+ = very slow germination.
2+ = slow germination.
3+ = slightly retarded germination.
4+ = normal germination.
0 = no germination.

TABLE 3.—*Effect of dry heat on germination of tomato seed*

Temperature, °C.	Control	50	50	55	55	60	60	65	65	70	70	75	75	80	80	80	85	85	95
Exposure, hours	.....	24	48	24	48	24	48	24	48	24	48	24	48	1	10	24	1	15	1
Per cent germination	80	61	65	84	23	64	74	73	10	51	12	40	55	53	50	51	60	40	0

for 72 hours, "the germination being more or less retarded, according to the temperature and time of exposure."

The results shown in table 3 show that tomato seed (2 years old) can withstand prolonged dry-heat treatment.

Dried on the surface of cover slips, the organism can remain alive for 2 hours at room temperature and for 10 minutes at 50° C.

Tomato seed[2] obtained by crushing ripe fruits from canker-diseased plants and subsequently by air-drying and storing at room temperature in the laboratory, for several months, were subjected to different treatments as indicated in table 4. Seeds treated with chemicals were afterwards washed in distilled water and then germinated in flats of steam-sterilized soil. The young plants, with 2 or more true leaves, were transplanted into large boxes of steamed soil, using about 500 plants of each lot (Table 4).

The canker organism is spread by the topping knife and by handling during transplanting. Thus, a few diseased plants may serve as a focus of infection, since a knife contaminated by cutting through or handling one diseased tomato plant is capable of infecting at least 30 healthy plants. The hot water treatment at a temperature of 53° C., effective in killing the tomato-canker bacteria, is known also to be injurious to the seed (10).

The writer's experiments with alcohol, alcoholic and aqueous solutions of malachite green, brilliant green, rosaniline hydrochloride, and dry heat show that the bacteria may be successfully eliminated by these methods. The writer noticed no ill effects on tomatoes in field plantings of either alcohol or dry-heat-treated seed. No field data on malachite, brilliant green, or rosaniline solutions are available, but greenhouse tests revealed no injury on plants after such treatments.

TABLE 4.—*Percentage of cankered tomato plants grown from treated and untreated seed collected from diseased plants*

Treatment		Percentage of plants diseased
Material and concentration	Duration	
Brilliant green 1: 1000 in water	60 minutes	2.9
Brilliant green 1: 1000 in 30% alcohol	60 minutes	1.5
Malachite green 1: 1000 in water	60 "	4.3
Malachite green 1: 1000 in 30% alcohol	60 "	0.7
Alcohol, 30%	60 "	0.0
Check (contaminated, untreated)	.....	26.0
60–61° C. (dry heat)	24 hours	0.0
65–68° C. (dry heat)	24 "	0.0

[2] Professor J. B. Kendrick kindly supplied the writer with some highly contaminated seed during the course of these studies.

SUMMARY

White, pink and rough variants of *Phytomonas michiganensis* (E.F.S.) Bergey *et al.* were studied and compared with the normal strain, physiologically and pathogenically. The variants are slightly different from the normal strain in their reactions on media and are less pathogenic.

Nicotiana glutinosa L. and *Cyphomandra betacea* Sendt., were successfully infected.

The following insects, *Myzus persicae* Sulz., *Lygus pratensis* L., *Thrips tabaci* Lind., *Heliothrips fasciatus* Perg., and *Diabrotica duodecimpunctata* Fabr., did not transmit the disease under greenhouse conditions. *Phytomonas michiganensis* could not be recovered from their mouth parts and internal organs after feeding on diseased plants.

Higher percentages of infection were obtained when the plants were topped with a contaminated knife than when punctured with a contaminated needle. The knife cuts remain susceptible to invasion 72 hours.

Unwounded plants did not develop the disease when sprayed with a heavy suspension of *P. michiganensis* and incubated in a moist chamber.

Brilliant and malachite green dyes are effective in killing the pathogen of tomato canker in aqueous and alcoholic solutions. Soaking tomato seed for more than 60 minutes in 5 per cent and 30 per cent alcoholic solutions of brilliant green, malachite green, and rosaniline hydrochloride, as well as in water solutions of these dyes, is not injurious.

Dry heat did not injure tomato seeds exposed to temperatures high enough to kill the pathogen. Dry seeds can withstand air temperatures up to 85° C. for 15 hours. This, however, reduced germination.

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GREENHOUSE METHOD FOR TESTING DUST SEED TREATMENTS TO CONTROL CERTAIN CEREAL SMUTS[1]

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COMMITTEE ON STANDARDIZATION OF FUNGICIDAL TESTS

(Accepted for publication December 15, 1943)

The principal causes of variability in the results obtained at times by different workers in cereal smut control with the same chemical dusts may be summarized as follows:

1. Relation of host and pathogen; differences in the relative susceptibility of the varieties or the virulence of the smut fungus strains used.
2. Method of inoculation; the spore dosage employed and the method of applying the inoculum to the seed (8, 9, 10).
3. Application of fungicide; amount of seed used, type of apparatus for applying the dust, thoroughness of application, and the period and conditions of storing the treated seed (5).
4. Environmental conditions between sowing and emergence; depth of sowing and compactness, moisture content, temperature, and reaction of the soil (1, 2, 7, 8, 12, 13, 16).
5. Post emergence conditions; this phase has not been entirely worked out but it has been shown that smut development in cereals may be influenced by certain conditions after emergence, chiefly temperature and perhaps moisture and length of day. It is thought these conditions may affect the development of the fungus until after it reaches and is established in the growing point of the host plant (7, 17, 18, 19).

The following procedures for use in greenhouse studies have been prepared primarily for testing commercial materials that have been placed on the market and also promising new fungicides upon which sufficient preliminary research has been done by the originators. They are not intended for use in developing new fungicides except as a final test of these in comparison with standard materials already on the market. In the earlier stages of developing new fungicides, the technique must necessarily be modified to meet the varying conditions.

BUNT OF WHEAT (TILLETIA TRITICI (BJERK.) WINT. AND T. LEVIS KÜHN)

Inoculum. Use spores from fully mature bunted heads collected from the crop of the current season; in dry areas spores from the previous crop may be substituted. Dry the bunt balls thoroughly and then break them up by rubbing through a 20-mesh sieve, followed by a series of finer sieves, the last one being 60-mesh. Keep this inoculum in a loosely stoppered bottle or jar at 5° to 10° C. until it is to be used.

Host. Use a highly bunt-susceptible variety of spring wheat, along with a physiologic race of the bunt organism especially pathogenic to it. The variety Ulka (C.I. 11478) is susceptible to almost all physiologic races and *Tilletia levis* race 3 is widely distributed; accordingly, these are recommended as standard.[2]

Inoculation. In order to inoculate the wheat, use a spore dosage of 1 to 200, *i.e.*, 5 grams of 60-mesh spore material to 1,000 grams of clean seed (136 grams of smut per bushel). Shake the seed, with the inoculum applied, in a tightly closed, half-filled container for one minute, by which time the seed should be thoroughly blackened. Sift the seed lightly to remove any excess spores.

Application of Fungicide. If the seed sample used in testing dust fungicides is too small, much of the proportionately smaller amount of chemical dust will adhere to the

[1] Reprints may be obtained at 10 cents each from the Committee Chairman, Boyce Thompson Institute, Yonkers 3, New York.

[2] A limited supply of seed grain and inoculum may be obtained through the courtesy of the following investigators:
Ulka wheat—R. H. Bamberg, Montana Agr. Exp. Station, Bozeman, Montana.
Anthony oats—M. B. Moore, Agr. Exp. Station, University Farm, St. Paul, Minn.
Odessa barley—C. S. Holton, Agr. Exp. Station, Pullman, Washington.
Odessa barley—V. F. Tapke, Bureau of Plant Industry Station, Beltsville, Md.
Tilletia levis, race 3—C. S. Holton.
Ustilago avenae—C. S. Holton.
Ustilago hordei, race 6, and *U. nigra*, race 4—V. F. Tapke.

container in which the treating is being done. Therefore, the container should first be "conditioned" by treating in it a sample of any kind of clean wheat, which can then be discarded, leaving the inside of the container coated with the fungicide to be tested. The size of the seed sample to be employed usually is governed by the quantity of available seed and the number of materials to be tested. A sample of 500 cc., *i.e.*, approximately 1/70 of a bushel, is recommended as a standard. This sample should be taken volumetrically by using a 500-cc. container. If the fungicide is to be applied at the rate of 1/2 ounce per bushel, a 500-cc. sample will require 0.2 gram (1/70 of 14.17 grams). Rates of 1, 2, 3, and 4 ounces per bushel will therefore call for 0.4, 0.8, 1.2, and 1.6 grams, respectively. This simplifies calculations involving bushel weights of different crops and also avoids a slight error in the rate of applying dust fungicides to samples of light, chaffy seed and plump, heavy seed of the same crop. The container should be large enough to hold more than twice the quantity of seed used so that it will be less than half-filled. After the sample of seed has been placed in the container, the proper quantity of fungicide, carefully calculated and weighed, is added and the container, with a dust-tight cover, is given a thorough shaking for two minutes. The treated seed is then placed in a cloth sack or open container and stored in a dry place for 48 hours when the amount necessary for greenhouse planting is removed. The remainder of the seed may be kept for later germination tests, or for testing further, under field conditions, particularly those materials eliminated in the greenhouse tests. Such field tests are advisable, however, only in areas where spring wheat is adapted.

Planting the Seed. Plant a portion of the treated seed 1½" deep in flats of soil, 50 per cent water saturated and with a reaction between pH 5.5 and 7.5 (14). A minimum of 5 replications of 100 seeds each for each test is recommended. Tamp the soil slightly after planting to bring it in close contact with the seed (1). Place the flats at 10° C. until after emergence and then remove them to a greenhouse held at 15° to 18° C. until the beginning of stem elongation or "shooting," when the temperature may be raised to about 25° C. If necessary, electric lights should be used to produce a photoperiod equal to that generally associated with the growing of spring wheat. If space at 10° C. is limited, the seed may be more thickly planted in smaller containers and, after emergence, the seedlings may be transplanted to flats or to the greenhouse bench. It goes without saying that in all such tests untreated, similarly inoculated seed should be planted under the same conditions for comparison.

Recording Results. Take data on emergence before the second leaf stage and on bunt infection at the stage when the diseased heads are most readily recognized. Percentages of infection should be based on head counts only. The significance of differences should be determined by statistical analyses; usually the analysis of variance (15) will be suitable for this purpose. In some cases transformation of data and cumulative error terms may be used to advantage (11).

LOOSE SMUT (USTILAGO AVENAE (PERS.) JENS.)

Inoculum. Since the two smuts of oats (*Ustilago avenae* and *U. levis*) are similar in life history and amenability to control by fungicides, the use of one species in testing dust fungicides is considered sufficient. Loose smut (*U. avenae*) is recommended as standard because of greater ease in preparing the inoculum.[3] Use spores from fully matured heads collected from the crop of the current season. Care must be taken to collect the heads of loose smut as soon as they appear and before the smut has been blown away. Sift the smut through a series of sieves, the last one being 60-mesh. Store the smut at 5° to 10° C. in a loosely stoppered glass container until it is to be used.

Host. Use the highly smut-susceptible spring variety Anthony (C.I. 2143).[4] This variety is recommended as a standard because, although not widely grown, it is susceptible to all commonly occurring physiologic races of *Ustilago avenae* and, therefore, the use of a particular race is not necessary.

Inoculation. Investigators have demonstrated that the smuts of oats may be perpetuated by spores on the seed, by spores and mycelium under the hull, and by mycelium in the epidermal cells of the pericarp (3, 6, 9, 12, 13). The infection due to spores on the seed is most easily prevented by seed treatment (9) while that due to inoculum under the hull is less amenable to control. It seems, therefore, that if a fungicide is to be thoroughly tested for oat smut control, it should be used on seed carrying inoculum under the hull (9). The use of a standard spore suspension and a partial vacuum in inoculating the seed is, therefore, recommended (4, 9, 10). In order to make a spore suspension, add 2 grams of 60-mesh spore material to a liter of distilled water and shake thoroughly until the spores are in suspension. Immerse 500 cc. of clean oats in one liter of this suspension in a high pressure desiccator and stir until the oats are thoroughly wet. To insure all kernels being beneath the liquid, a wire screen that fits the inside of the

[3] See footnote 2.

[4] See footnote 2.

vessel should be placed on the surface of the liquid. A wood strip an inch thick is fastened to its upper side. Install a vacuum gauge in the suction line and apply 25 inches of vacuum for 5 minutes, shaking the jar occasionally to promote the escape of air from beneath the glumes. After 5 minutes, release the vacuum and then apply it again for another 5 minutes. After again releasing the vacuum, drain the seed and spread it out on absorbent paper to dry for 24 hours to allow air to get under the glumes. Then place the seed in a one-half inch layer at 20° C. and 80 to 90 per cent humidity for 20 hours, after which it should be dried thoroughly with a fan and stored in a dry place until it is to be used.

Treating, Planting. The directions for treating and planting the seed are similar to those given for wheat, except that the temperature between planting and emergence should be from 18° to 20° C., the soil reaction should be about pH 7.4 (13), and the soil should be about 30 per cent saturated.

Recording Results. Data on emergence and on the percentage of smut are taken as described for wheat. Frequently, complete control of oat smuts in vacuum-inoculated seed is not obtained, even with dust fungicides that eliminate smut completely in naturally infested seed (9). In such cases the fungicidal efficiency of new materials being tested can be determined by comparing their performance with that of a standard fungicide known to be effective, and included in the same test.

COVERED SMUT (USTILAGO HORDEI (PERS.) KELL. AND SW.) AND BLACK OR SHALLOW-BORNE LOOSE SMUT (USTILAGO NIGRA TAPKE) OF BARLEY

Inoculum. The most prevalent and widely distributed physiologic races of the above smuts in the United States are race 6 of *Ustilago hordei* (16) and race 4 of *U. nigra* (21). Therefore, the use of these races is suggested as part of the standard procedure in testing dust fungicides for the control of these two barley smuts.[5] The inoculum should be collected and prepared for use as described for the smuts of wheat and oats.

Host. Odessa barley (C.I. 934) is highly susceptible to all known races of covered smut (16) and the shallow-borne loose smut (21). Therefore, this variety is recommended as a standard. It should be noted that this is not the commercially grown Odessa but the special strain labeled C.I. 934. This strain is highly susceptible also to the deep-borne loose smut, *Ustilago nuda,* which can be controlled only by the hot-water treatment. Because the heads of these two loose smuts closely resemble each other, seed free from *U. nuda* infection must be used to avoid confusion. Since the extreme susceptibility of Odessa (C.I. 934) makes it undesirable commercially, seed must be obtained from a station where increase plots are grown to maintain a soure of supply for experimental purposes.[6]

Inoculation. The inoculum of barley covered smut that is most effective in producing the disease and also most difficult to combat is that found beneath the hulls (20). Smut spores borne on the surface of the seed may be eliminated by copper carbonate, a fungicide that is generally considered unsatisfactory for controlling naturally induced covered smut of barley. Therefore, as in the case of the oat smuts, in order to test adequately a fungicide for the control of barley covered smut, seed should be used that carries abundant inoculum beneath the hulls. The same probably holds true for black loose smut (*Ustilago nigra*). The method of inoculating barley seed with either of the two smuts is the same as that described for the oat smuts.

Treating, Planting, Results. The treating and planting of the seed and the recording of the data are also similar to those for the oat smuts except that, for covered smut, the soil reaction may range from pH 6 to pH 7 (2) and for black loose smut the range appears to be still greater (7). A soil reaction of pH 6.5 and a temperature of 20° C. during emergence are suggested for both of these smuts.

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SEED TRANSMISSION OF SQUASH-MOSAIC VIRUS[1]

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(Accepted for publication December 30, 1943)

INTRODUCTION

There are several virus diseases common to squash, *Cucurbita pepo* L. var. *condensa* Bailey, along the coast and in the inland districts of southern California. Of these virus maladies, western cucumber mosaic and squash mosaic are the ones most frequently observed. This paper is concerned with squash mosaic.

The disease occurs in the Tijuana Valley on the Mexican border and has been observed in the San Francisco Bay area, but is perhaps most often found in the coastal districts, from San Diego to Santa Maria. Though not so commonly observed inland, squash mosaic has been found in the western portions of Riverside and San Bernardino counties, but not as yet in the further inland desert areas of the Coachella and Imperial valleys.

In southern California, squash mosaic is present, but is relatively unimportant early in the growing season. As the plants begin to mature fruit, the disease may be found more frequently. Toward the close of the growing season it is omnipresent and responsible for considerable loss to the grower. In the San Francisco Bay area, however, squash mosaic is very important early in the season, especially in fields near the foothills; it has been suggested that the incidence of early infection may be closely related to the occurrence of overwintering forms of *Diabrotica* spp., which may serve as a source of the virus.

Kendrick (8) described a mosaic disease of muskmelons, *Cucumis melo* L., and proved the disease to be seed-transmitted. Infection percentage varied from 0.0 to 2.13, with a mean percentage infection of 0.25. Freitag[2] has identified the virus with which Kendrick worked as the squash-mosaic virus affecting muskmelons. Freitag (6, 7), in two abstracts dealing with squash mosaic, presents the properties of the virus, establishes insect transmission by the western spotted cucumber beetle, *Diabrotica soror* Lec., the western striped cucumber beetle, *D. trivittata* (Mann.), and 5 species of aphids. He found that the disease could be produced by inoculation in 15 species of plants distributed in 11 genera within 4 families.

Few references reporting seed transmission of cucurbit viruses are to be found in the literature. McClintock (10) indicates that cucumber-mosaic virus may be seed-borne. Doolittle and Gilbert (4) report seed transmission of the cucumber-mosaic virus by the wild cucumber, *Echinocystis lobata* (Michx.) Torr. and Gray. Doolittle (3) concludes that only one plant out of 10,000 grown from seed collected from infected fruits devel-

[1] Paper No. 502, University of California Citrus Experiment Station, Riverside, California.

[2] In correspondence with the writer, March 22, 1943.

oped mosaic symptoms not attributable to outside infection. He further states that "it is doubtful whether cucumber mosaic is seed-borne to any considerable extent, but it seems possible that it may occur in rare cases, and the results so far obtained have left the problem still open, warranting further investigation." Doolittle and Walker (5) present data showing one case of apparent mosaic seedling infection in approximately 22,000 cucumber plants; negative results were secured with mosaic-infected squash, muskmelon, and pumpkin. Bewley and Corbett (2) believe the cucumber-mosaic virus to be readily seed-transmitted. Mahoney (9) presents evidence of seed transmission of an unestablished virus in inbred lines of muskmelons; infection percentage varied from 14.3 to 33.3 and averaged 15.6. Ainsworth (1) states that yellow-mottle mosaic of cucumber, *Cucumis sativus* L., has

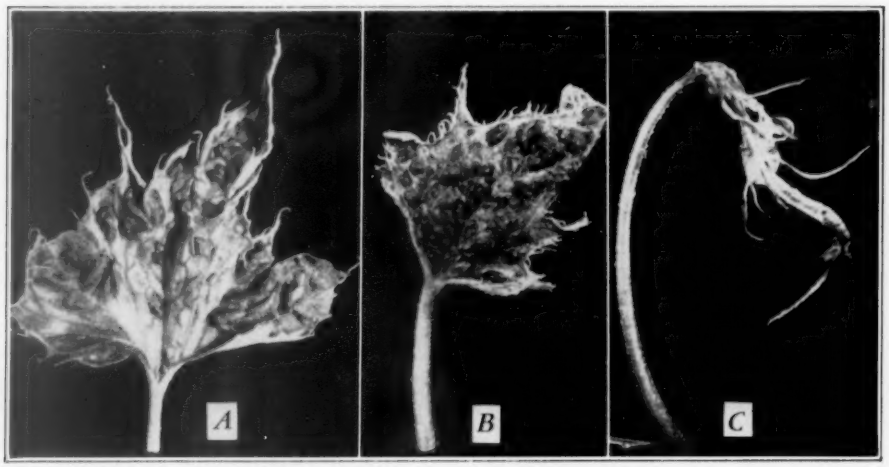

FIG. 1. Leaves of yellow crookneck squash, showing symptoms produced by the squash-mosaic virus. *A*. Distorted, rugose, mottled condition typical of the disease. *B*. Extreme rugosity. *C*. Leaf structure reduced largely to veinous system.

been shown to be seed-transmitted and believes that the yellow-mosaic and green-mosaic viruses are also seed-transmitted, but he gives no supporting data.

SYMPTOMATOLOGY

The most conspicuous and spectacular symptom of squash mosaic is the presence of filiform leaves; infected leaves are frequently reduced to only the veinous system, with patches of green mesophyll (Fig. 1). Leaves may be distorted, rugose, and mottled with dark-green raised areas; little if any yellowing occurs. Fruits from infected vines are malformed, the usual even contour broken by raised, more or less domelike areas, $\frac{1}{4}$ to $\frac{1}{2}$ inch in diameter; very often the affected areas are yellow or mottled (Fig. 2). Infected plants fail to grow with normal rapidity, set fewer fruits than healthy plants, yet rarely die as a direct result of squash-mosaic infection.

SEED SELECTION AND PLANTING

In order to determine whether or not the squash-mosaic virus is seed-transmitted, mature fruits showing typical fruit symptoms were collected from badly diseased vines. Collections were made in San Diego and Orange counties. Three varieties were chosen: White Bush Scallop, Yellow Crookneck, and Italian Marrow or Zuchini. Seeds were extracted by scooping them out of the fruits and washing thoroughly in water. Many of the infertile and light seeds were floated off during the washing. The remaining

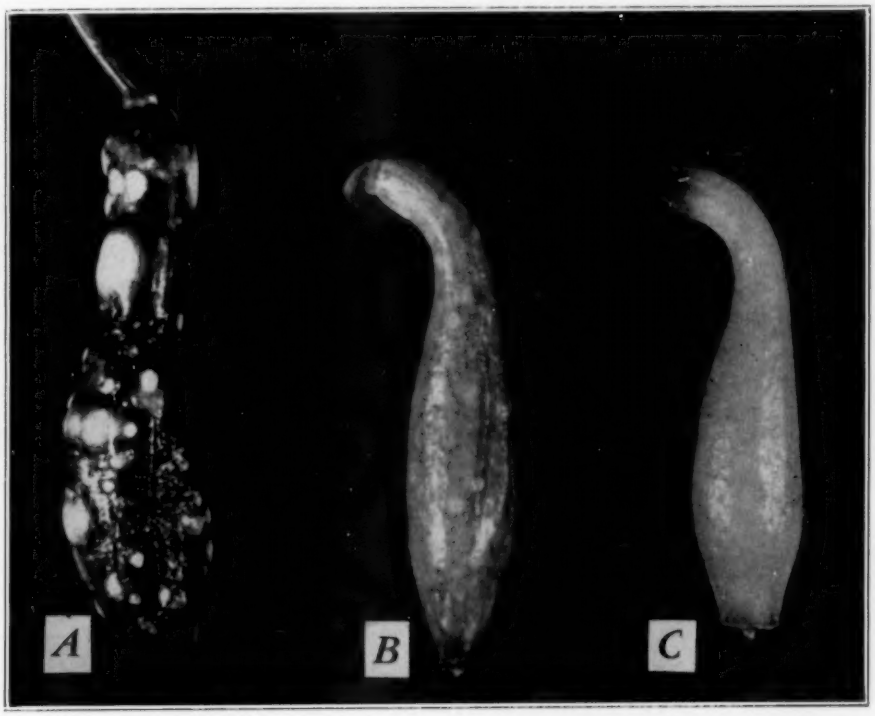

FIG. 2. Immature fruits of yellow crookneck squash, showing effect of infection by the squash-mosaic virus. *A*. Severe symptoms. *B*. Mild symptoms. *C*. Healthy fruit.

seeds were then dried and winnowed, and the light, poorly filled, deformed seeds were segregated from the heavy, well-filled seeds.

Seeds were sown in sterilized soil contained in sterilized redwood seed flats, which were placed on benches in a glasshouse. Separate sowings were made of the light, poorly filled, deformed seed and of the heavy, plump seed. Plantings were made on different dates, at similar intervals, over a period of about 3 years. The section of the glasshouse used was so designed and constructed as to exclude, as far as possible, any ingress of insects. The glasshouse was fumigated with nicotine subsequent to seeding and again after emergence of the seedlings.

RESULTS

Seedlings first made their appearance 5 days after irrigation, but the majority emerged 7 days after watering. No symptoms of the disease were

TABLE 1.—*Percentage transmission of squash-mosaic virus through seed collected from infected squash fruits on August 14, 1940, and planted at intervals over a 3-year period*

Age of seeds, in weeks	Total number of plants per sowing	Diseased plants	
		Number	Per cent
Heavy, well-filled seed			
4	425	1	0.23
7	285	0	0.00
11	351	0	0.00
19	1124	3	0.27
23	1091	1	0.09
32	540	2	0.37
35	639	1	0.16
44	873	0	0.00
51	1420	2	0.14
60	1563	2	0.13
69	1559	3	0.19
72	1492	2	0.13
76	1261	3	0.24
83	1202	3	0.25
91	1947	5	0.26
95	1572	3	0.19
98	1503	2	0.13
103	1380	1	0.07
109	1729	2	0.11
112	1294	3	0.23
121	876	0	0.00
130	981	1	0.10
138	735	0	0.00
145	1611	2	0.12
149	1564	3	0.19
156	1477	2	0.13
159	1372	0	0.00
Mean	.....	.....	0.14
Light, poorly filled, deformed seed			
4	228	2	0.88
10	1231	17	1.38
22	970	6	0.62
31	361	8	2.22
50	473	3	0.63
61	1076	13	1.21
74	1370	14	1.02
83	525	4	0.76
90	1209	16	1.32
93	1068	7	0.65
102	959	6	0.62
111	805	4	0.50
120	804	5	0.62
131	1253	14	1.12
140	1623	18	1.11
155	537	4	0.74
Mean	.....	.....	0.96

observed on the cotyledons, though symptoms were usually conspicuously present on the first leaf. Symptoms could be observed immediately upon

the unfolding of the leaves, while they were still quite small. In very few instances were symptoms absent from the first leaf and yet present on the second leaf; rarely did the disease develop on the third leaf and not on the preceding ones.

After the plants had grown for 8 weeks, counts were made to determine the total number of plants and the number diseased. Results of these counts are shown in table 1.

DISCUSSION

The virus causing squash mosaic is definitely seed-transmitted. The relatively low percentage of infected plants arising from infected seed is in harmony with the low amount of the disease usually present in early field plantings. The presence of these infected seedlings provides an excellent and immediate reservoir of the virus, which then can be readily and rapidly spread throughout the planting, either by mechanical means or by insect vectors. This apparently transpires in the field. Fields in which an effort is made to reduce the populations of aphid and *Diabrotica* spp. invariably show less effects of the disease.

Seed stocks should be acquired only from fields free from squash mosaic. The fact that a higher percentage of the disease is transmitted by the light, poorly filled, deformed seed than by the heavy, well-filled seed is of interest and of practical import. If a seed stock is collected from an acreage known to harbor the disease, careful winnowing will remove the majority of irregularly filled seeds and thereby reduce the total amount of the disease that would normally be carried through such seed.

The data given in table 1 show that there was no apparent difference in the percentage transmission in plantings made soon after the seed was harvested and in the final planting, about 3 years later. The virus may be carried by seed at least 3 years old, with no decrease in the percentage of disease transmission.

The fact that a higher percentage of virus is carried by malformed seed than by normal seed, as well as the length of time that the virus remains active, indicates that the latter may be borne internally and not on the surface of the seed coat.

The percentage transmission of squash-mosaic virus through seed of melon and of squash seems very similar. The mean percentage given by Kendrick (8) for muskmelon (0.25 per cent) is of the same magnitude as that determined by the writer for squash (0.14 per cent), for seed of good quality. A higher percentage transmission is prevalent in squash seed of poorer quality (0.96 per cent).

Mahoney (9) probably was dealing with a different virus disease of cucurbits than that discussed herein. The percentage seed transmission that he reports (maximum, 33.3 per cent) is considerably above the values given by Kendrick or the writer (maxima, 2.13 and 2.22 per cent, respectively).

SUMMARY

The symptoms of the squash-mosaic-virus disease are described. The virus is demonstrated to be seed-transmitted. Poor-quality squash seed—that is, light, poorly filled, deformed seed—proved to carry a higher percentage of the causal virus than good-quality, heavy, well-filled seed taken from the same seed population. The virus remains viable in 3-year-old seed, and there is no apparent difference in the percentage of seed transmission of the virus in seed samples sown shortly after harvesting or about 3 years later.

The disease may be controlled to some extent by harvesting squash seed from fields that are free of the disease, and, perhaps, by planting in areas not in close proximity to vector-breeding grounds. If seed is taken from fields known to be infected with squash mosaic, the percentage of seed transmission may be materially reduced by careful winnowing.

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PREVALENCE AND DISTRIBUTION OF STRIPE SMUT OF POA PRATENSIS IN SOME PASTURES OF PENNSYLVANIA[1]

K. W. KREITLOW AND W. M. MYERS[2]

(Accepted for publication January 1, 1944)

Observations by Pammel (6), Clinton (2), Osner (5), Davis (3), and others have emphasized the widespread occurrence of stripe smut of grasses caused by *Ustilago striaeformis* (West.) Niessl. Only limited information is available, however, regarding the prevalence and importance of this smut in Kentucky bluegrass pastures. Allison and Chamberlain (1) reported abundant infection of bluegrass pastures in the vicinity of Richland Center, Wisconsin. Every pasture was infected, and in many cases damage was extensive. Wellhausen, *et al.* (7), examined bluegrass pastures in West Virginia and Pennsylvania and found smut very prevalent. From a total of 504 sod plugs taken at random from different pastures in West Virginia, 15 per cent were diseased. Re-examination of certain pastures revealed an estimated range in smut infection from a trace to about 25 per cent.

Davis (3) found that more than 90 per cent of smutted plants fail to survive periods of drought. Observation has shown that prevalence of smut varies with season of year so that many more diseased plants are noticeable during spring and fall than during hot summer months when droughts generally occur. It has been observed further (4) that bluegrass plants, apparently free of stripe smut when collected, often developed smutted shoots when they were maintained under favorable conditions in a greenhouse. This indicated that a superficial examination of pastures for prevalence of smut in different seasons might be misleading, since some diseased plants could be symptomless at the time of examination, but harbor latent infections of the pathogen that would become manifest under favorable conditions. This suggested that smut infection among plants of *Poa pratensis* may be far more extensive than is generally realized.

MATERIALS AND METHODS

In a preliminary survey of more than 75 bluegrass pastures in Pennsylvania, the writers found very few that appeared to be free of stripe smut, while many bore extensive infection.

During the fall of 1942, each of 13 representative pastures in the vicinity of State College, Pennsylvania, was divided by estimation into 4 sections, and approximately 50 sod plugs, 1.5 inches in diameter, were collected at random from each section. The sod plugs were examined for smut on the day following collection, and the number of plugs containing smutted plants was recorded.

[1] Contribution No. 59, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in cooperation with the Northeastern States.

[2] Associate Pathologist and Geneticist, respectively.

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In order to preserve the plugs for further examination, those from each section of a pasture were placed in rows in a flat containing wet peat moss. Thus, each plug was kept separate and the plants therein were maintained under favorable growing conditions. Each of the 2600 plugs was examined for smut at 1-, 2-, 3-, and 5-month intervals. The plants in each plug were clipped periodically to maintain them at a height convenient for examination.

RESULTS

Smutted plants (Table 1) were obtained from each of the 13 pastures. At the time of sampling, the proportion of plugs with smutted plants varied for different pastures from 0.5 to 11.4 per cent. When the plugs were re-examined one month later, nearly all samples showed an increase in number of smutted plugs. The collections from some pastures contained more than twice as many smutted plugs as were detected at the first examination.

TABLE 1.—*Per cent of plugs with smut-infected plants at different periods of examination and total per cent smut*

Pasture No.	Months after collection					Total[a]
	0	1	2	3	5	
70	7.6	12.0	18.1	19.5	17.7	30.0
71	4.9	9.3	14.2	13.0	11.7	25.9
72	4.0	4.0	5.0	6.5	5.5	10.0
73	0.5	1.5	2.1	2.0	1.5	4.5
74	5.5	6.0	9.0	11.0	10.0	16.5
75	11.4	15.3	18.1	18.7	14.9	31.0
76	3.9	9.9	20.8	25.3	25.2	34.4
77	5.5	8.4	13.4	9.9	14.9	21.8
78	11.1	10.5	13.5	17.0	13.0	23.0
79	10.6	12.6	15.2	14.2	12.7	23.9
80	9.0	16.3	18.2	17.2	19.1	29.5
81	3.9	7.7	7.7	7.8	8.3	14.6
82	2.0	5.0	5.0	4.5	4.6	11.0

[a] Total per cent smutted plugs recorded during the entire observation period.

There was no evidence that increase in number of smutted plugs from one examination period to the next was due to spread of infection among plugs in the flats. This was demonstrated by plotting the location of smutted plugs after each examination. If transmission of smut were taking place, one would expect the healthy plants in plugs adjacent to diseased plants to be the first to show symptoms of infection. However, the location of additional plugs containing smutted plants appeared to be random.

When the plugs were examined 2 and 3 months later, a further increase in the number of smutted plugs was found in some collections. This indicated that most of the smut present among random samples from different pastures was detected by growing the plugs for at least 3 months under favorable conditions in the greenhouse. At the last examination, the collections from most pastures showed a decrease in number of smutted plugs;

however, 1.5 to 25.2 per cent of plugs from the different pastures still contained smutted plants.

The decrease in smut observed in some collections was due to death of the diseased plant or plant part in some of the plugs. Since only healthy plants or at least plants showing no symptoms of disease were left, classification of such plugs was changed from diseased to healthy.

A measure of total smut present among collections of plugs from each pasture was determined by adding together all plugs recorded as smutted during the 5-month observation period. This figure included plugs in which smutted plants succumbed or those that contained plants that lost their smut symptoms, as well as smutted plants in plugs that were designated as healthy at earlier examinations. Since a record was kept regarding the status of

TABLE 2.—*Distribution in per cent of smutted plugs within different parts of four representative pastures*

Pasture No.	Section	Months after collection				
		0	1	2	3	5
72	1	4.0	4.0	4.0	6.0	9.0
	2	12.0	10.0	14.0	20.0	14.0
	3	0.0	0.0	0.0	0.0	0.0
	4	0.0	2.0	2.0	0.0	0.0
73	1	0.0	1.9	0.0	3.8	1.9
	2	2.1	2.1	8.3	4.2	2.1
	3	0.0	0.0	0.0	0.0	0.0
	4	0.0	2.0	0.0	0.0	2.0
76	1	3.8	5.8	13.5	17.3	25.0
	2	3.9	9.8	19.6	31.4	23.5
	3	0.0	8.2	18.4	22.4	22.4
	4	8.0	16.0	32.0	30.0	30.0
80	1	8.2	10.2	12.2	20.4	16.3
	2	0.0	4.0	8.0	6.0	4.0
	3	3.8	18.9	26.4	26.4	30.2
	4	24.0	32.0	26.0	16.0	26.0

each plug at each examination, any plug that had displayed a smutted plant at any time during the observation period was recorded as smutted. The data so recorded are shown in the last column of table 1 and illustrate the extensive smut infection present in the pastures sampled. The least proportion of smutted plugs recorded was 4.5 per cent from pasture No. 73. The greatest proportion of smutted plugs collected, 34.4 per cent, came from pasture No. 76. Of the 13 pastures sampled, 8 yielded more than 20 per cent smutted plugs.

DISTRIBUTION OF STRIPE SMUT IN SOME PASTURES SAMPLED

The distribution of stripe smut in pastures was studied by recording separately the number of smutted plugs collected from the 4 parts of each pasture. The data from table 2 show that some pastures varied appreciably in the amount of smut present in different areas. For example, sections 3 and 4 of pasture No. 72 showed no smut at the time they were sampled, while

sections 1 and 2 of the same pasture contained smut to the extent of 4.0 and 12.0 per cent, respectively. The number of smutted plugs in sections 1 and 2 increased eventually to a maximum of 9.0 and 20.0 per cent. Similarly, there were wide fluctuations in different parts of pasture No. 80. At the first examination, the number of smutted plugs from different sections of this pasture varied from 0 to 24.0 per cent. At the last examination, the variation was from 4.0 to 30.2 per cent.

The results from pastures 73 and 76 (Table 2) are illustrative of those obtained from pastures bearing a fairly uniform distribution of smut. Despite some fluctuation in number of smutted plugs at different examinations, the final examination revealed a rather uniform amount of smut among the samples collected from these pastures.

DISCUSSION

From the data presented, one may conclude that prevalence of stripe smut of *Poa pratensis* in some pastures of Pennsylvania is much greater than superficial examinations would ordinarily disclose. It is well known that the amount of smut present during different seasons fluctuates, depending on weather conditions. Under favorable growing conditions of spring and fall, considerable numbers of smutted plants are noticeable. That the amount of smut visible in a bluegrass pasture at any one time is not necessarily representative of the total amount present was demonstrated by the marked increase in number of sod plugs containing smutted plants when the samples collected from different pastures were grown under favorable conditions in a greenhouse. The increased number of smutted plugs was apparently due to expression of symptoms among plants that were symptomless at the time they were collected and to dormant infections among some plants.

The high percentage of smutted plugs collected from some of the bluegrass pastures leaves little doubt that stripe smut may be important in reducing yields. The observed decrease in prevalence of smut during hot summer months is probably due to death at least of the above-ground parts of many diseased plants. This may account, to some extent, for the so-called "drying-up" of bluegrass pastures during the summer season. With the onset of unfavorable environmental conditions for the host, stripe smut might weaken plants to a point where they would succumb to the infection or be predisposed to invasion by other pathogens.

The pastures studied in these investigations varied in size from a few to many acres. Within some of the pastures there was a wide range in topography, soil type, fertility, and density of Kentucky bluegrass. Within the limits of the sampling in this investigation, there was no apparent consistent relationship between these factors and variations in prevalence of smut infection. It is possible that more extensive investigations would reveal such relationships. Other factors that may have influenced variations within pastures are age of pasture, distribution of susceptible strains of Kentucky

bluegrass, and management practices and other conditions affecting the spread of the pathogen and survival of diseased plants. These factors also may have been important in conditioning differences between pastures.

The results of these investigations emphasize the importance of a further study of the life history, epidemiology, ecology and genetics of the pathogen causing stripe smut.

SUMMARY

Examination of *Poa pratensis* plants in sod plugs collected from different parts of each of 13 representative pastures in Pennsylvania revealed that stripe smut caused by *Ustilago striaeformis* was present at the time of sampling to the extent of 0.5 to 11.4 per cent.

The sod plugs from each pasture were maintained separately and observed periodically. In most cases, there was an increase in number of plugs containing smutted plants. This was attributed to expression of symptoms among plants that were symptomless at the time they were collected and to dormant infection.

The total number of plugs recorded as containing smutted plants during a 5-month observation period varied for different pastures from 4.5 to 34.4 per cent.

Observations on distribution of stripe smut in different parts of pastures revealed that some contained a fairly uniform infection over the entire pasture while others contained variable amounts for different areas.

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CARROT BACTERIAL BLIGHT AS IT AFFECTS THE ROOTS

P. A. Ark and M. W. Gardner

(Accepted for publication January 3, 1944)

A scab disease of carrot roots, serious in California (1) has been found to be caused by the bacterial blight organism, *Phytomonas carotae* (Kendrick) Bergey *et al.*, 1939, described by Kendrick (2) in 1934 as the cause of a disease of the leaves and umbels. The root phase of this disease is probably identical with a scab reported as an important market blemish in California-grown carrots in 1936 by Ramsey (3, p. 86) and Ramsey and Wiant (4, p. 43, and Pl. 13–B). It was first observed in California in 1937 in the Santa Maria Valley, and is prevalent near Soledad in the Salinas Valley in irrigated fields of sandy soil, cropped repeatedly to carrots.

The bacterial-blight organism has been isolated from the root lesions, and its pathogenicity proved by atomizer inoculation of carrot foliage. Root infection was obtained with this organism in carrot plants grown from sterilized seed in flats of sterilized soil to which a suspension of the organism had been added.

SYMPTOMS

Infection may occur at any point on the surface of the root (Fig. 1, A, D). Late infection of fairly large roots causes rather shallow, scabby lesions, often very numerous (Fig. 1, A, B, C, D). Young lesions are very small, brown or maroon spots, which may develop into slightly raised pustules or slightly sunken, laterally elongated craters with flakes of black necrotic tissue at the center (Fig. 1, A, B, Fig. 2, B). These lesions often bear gray masses of bacterial ooze. Examination of washed roots with a hand lens often reveals another type of incipient lesion associated with this disease, a shallow, water-soaked or greasy fleck 2 or 3 mm. in diameter, involving only the epidermis (Fig. 2, B). Such lesions may develop a brown necrotic spot in the center.

Larger lesions are elongated laterally (Fig. 1, A, B, C, Fig. 2, A, C). The necrotic tissues in the lesion crack open and a copious bacterial exudate embedding soil particles and fragments of the black necrotic tissue is largely responsible for the raised, black, scabby character of the lesion. When the roots are washed the lesions emit fresh gray masses of bacterial exudate. Kendrick (2) pointed out this tendency of the blight lesions on the umbels to ooze copiously.

Because of its interference with normal root enlargement, early infection may result in a sunken area with a scab (Fig. 2, F) or blackened canker (Fig. 2, D) at the center or a healed-over pocket of blackened necrotic tissue (Fig. 2, E, F). These internal or buried lesions in fairly normal appearing roots are particularly objectionable because such roots cannot be culled out in the field or packing sheds. Occasionally large, rough, sunken cankers

are formed by coalescence of early lesions (Fig. 2, D). Early infection may be fairly deep-seated, and, although usually arrested and overgrown, may be fatal to young plants.

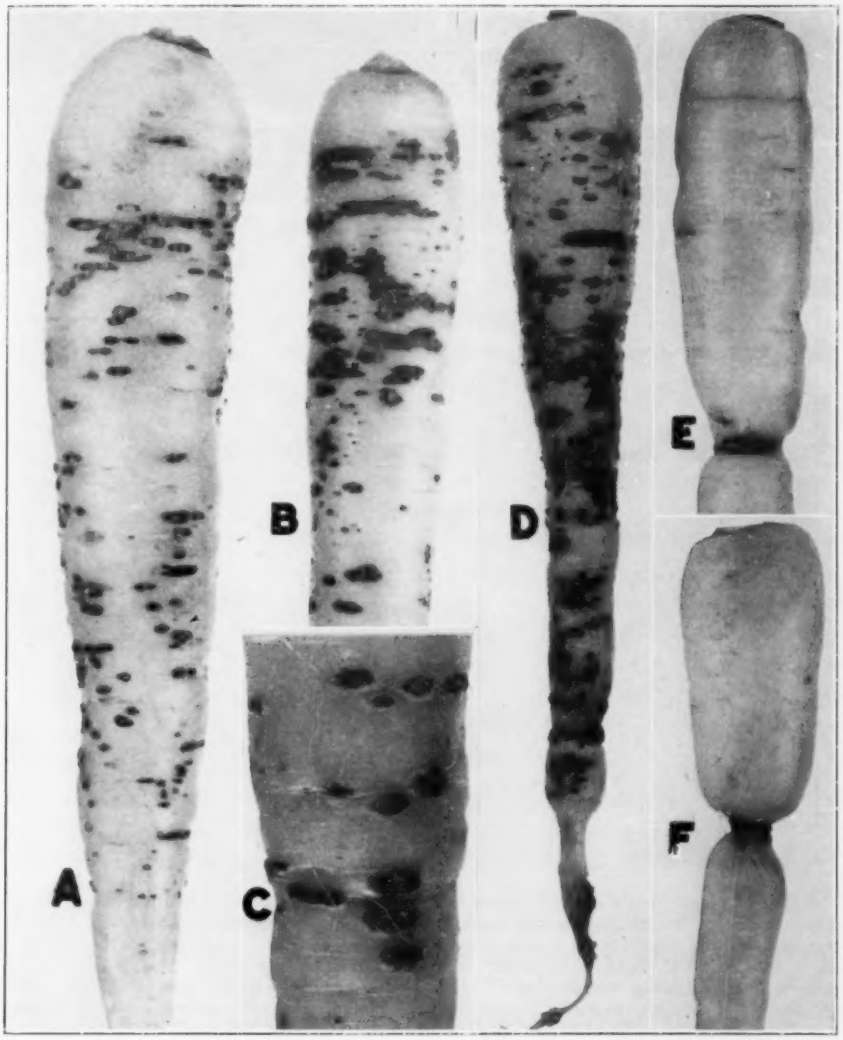

FIG. 1. Root symptoms of carrot bacterial blight. A to D. Raised scabby lesions, elongated laterally. In B and D some very small lesions are shown. E and F. Encircling lesions and root constriction resulting from early infection.

Frequently early infection results in a sharply sunken encircling constriction of the root, which may cause it to break in two when pulled (Fig. 1, E, F). Secondary infection of blight lesions by rot-producing fungi is of frequent occurrence.

Internal infection of the crown of old roots used for seed production has

been found in the form of discolored strands (Fig. 2, G) proceeding downward, presumably from previously infected leaves.

PERSISTENCE OF ORGANISM IN THE SOIL

In the Soledad region where root infection is prevalent, carrots for eastern shipment have been grown more or less continuously and at all seasons of the year, some fields having a history of 4 carrot crops in 3 years. Soil

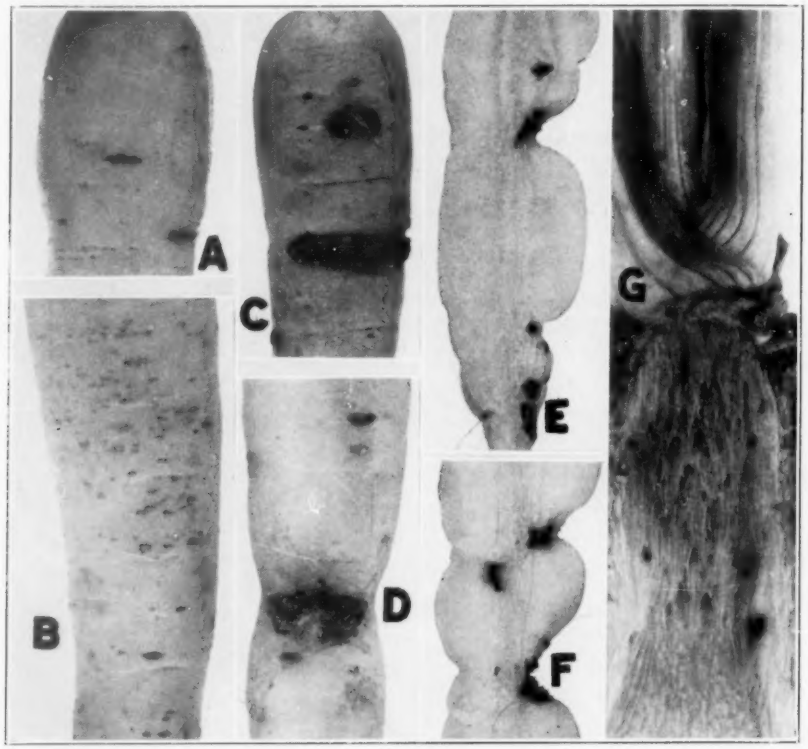

FIG. 2. Root symptoms of carrot bacterial blight. A. Laterally elongated scab lesions and very small incipient lesions. B. Two types of incipient lesions, laterally elongated, sharply delimited, black specks and freckle-like, skin-deep, watersoaked or greasy blotches. C. Large, laterally elongated, encircling scab. D. Sunken canker, resulting perhaps from coalescence of early infections. E and F. Longitudinal sections through root showing constrictions and internal pockets of black necrotic tissue resulting from early infection. G. Longitudinal section of crown of old seed root showing dark strands of necrotic infected tissue that extend downward from infected leaf bases.

conditions are evidently favorable for root infection throughout most of the year, since none of the crops representing different dates of planting seem to escape. On the other hand, foliage infection in these fields has been observed only in the spring on crops exposed to the winter rains and is believed to have little or no current relation to root infection.

Kendrick (2) has shown that foliage infection may come from the soil. That the soil in the Soledad carrot district is heavily contaminated with the

blight bacteria has been proved by the abundant root infection obtained on carrots grown from hot-water-treated seed in flats of soil brought in from diseased fields to the greenhouse. In fact, infection was obtained after the soil had been in dry storage for 12 months.

The copious oozing of the bacteria from the root lesions and the dangerous harvest practice of leaving the culls in the field to be plowed under provide for abundant soil infestation.

The disease is much worse in fields repeatedly cropped to carrots. For example, counts made at Soledad in January, 1943, showed conspicuous infection on 12 per cent of the roots in new soil as compared with 50 per cent in old carrot soil. These plots were planted with sterilized seed, and the presence of the organism in the soil not previously in carrots may possibly be explained by the deposits of wind-blown, contaminated soil from old carrot fields nearby. A very strong afternoon wind that drifts the soil is a daily phenomenon in that district at certain seasons.

SEED DISSEMINATION AND SEED DISINFECTION

Kendrick (2) showed that bacterial blight occurred abundantly in the umbels of carrots grown for seed and that artificially contaminated seed gave rise to infected seedlings. Seed from infected and healthy umbels was collected in the Delta region in June, 1940. Seed from infected umbels was planted in flats of sterilized soil in the greenhouse in late October and among about a thousand seedlings examined, 4.3 per cent showed foliage infection. In field plots planted with this seed in July, 1940, the plants were grown until they bore seed. In September, 1941, foliage and umbel infection was found on 8 of the 199 plants grown from seed collected from diseased umbels, whereas no disease developed among the 236 plants grown from seed collected from healthy umbels.

The same carrot seed from infected umbels was used to test various methods of seed disinfection. In greenhouse tests in sterilized soil, started in January, 1941, 9 per cent of the several hundred plants grown from untreated seed were infected, whereas no infection occurred among a similar number of plants grown from seed treated in hot water, 52° C., for 10 minutes. Field plots were planted in Berkeley in the spring of 1941 in soil not previously in carrots, and disease counts were made in May. In the check plot planted with seed dipped in water, 47 per cent of the seedlings were infected, whereas no infection occurred in the plots planted with seed treated for 10 minutes in hot water at 52° C. or for 10 minutes in a 1:1000 solution of mercuric chloride. In the field test previously mentioned in which the seed was planted in July, 1940, and the records taken over a year later, 4 per cent of the plants from unsterilized seed were infected, 3 per cent, where seed treated in a 1:1000 solution of mercuric chloride was used, and none where the seed treatment was hot water, 52° C., for 10 minutes.

Large field plots have been planted with hot-water-treated commercial seed at various times during 1942 and 1943, in the Soledad district, with no

indication of any injury from the treatment; but the presence of general soil infestation with the blight bacteria rendered the seed treatment ineffective against the disease.

SUMMARY

Black, scabby lesions on carrot roots are caused by the carrot bacterial blight organism, *Phytomonas carotae*. Deep constrictions and internal pockets of necrotic tissue may result from early infection.

The organism is harbored in the soil and the disease is serious in fields cropped repeatedly to carrots.

Hot-water treatment of the seed is effective against seed-borne infection.

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THE POTENTIAL IMPORTANCE OF RACE 8 OF PUCCINIA GRAMINIS AVENAE IN THE UNITED STATES[1]

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(Accepted for publication December 31, 1943)

Race 8 of *Puccinia graminis avenae* was sufficiently prevalent in 1943 to cause concern regarding its possible future effect on the performance of Vicland, Boone, Tama, and several other recently developed oat varieties, mostly derived from Victoria × Richland crosses. Because of their yielding ability and resistance to stem rust, crown rust, and smuts, these new varieties have been rapidly replacing older varieties of oats. They owe their stem-rust resistance to the Richland parent, one of the differential varieties used in identifying races of *P. graminis avenae*.

Although Richland is susceptible to 7 of the 13 races of *Puccinia graminis avenae* now known, none of them has hitherto been sufficiently prevalent in the United States to be of practical importance, and only 2 races (8 and 10) have been found commonly. Richland and the varieties that derived their resistance from it are not susceptible to races 2 and 5, the only races prevalent enough to be of practical importance during the past 15 or 20 years (3, 5, 6, 7, 8). Nevertheless, as pointed out by the writers and Cotter (6, 8), there have been indications recently that races 8 and 10 might increase sufficiently in prevalence to endanger those varieties with the Richland type of resistance.

The prevalence of races, expressed as percentages of total isolates for 1939 to 1943, inclusive, is shown in table 1.

Only 6 races were represented in more than 1,200 isolates during the past 5 years; and, for practical purposes, the number may be considered as 4, since races 2 and 5 may be combined; likewise 8 and 10. Races 2 and 5 differ only in their effect on the variety Jostrain, race 2 producing type 4 and race 5 producing type X. There is a similar difference between races 8 and 10 on Jostrain. When the combinations are made, it is apparent that races 2 and 5 comprised most of the rust during the past 5 years, races 7 and 12 were almost negligible, and races 8 and 10 were present in small but appreciable amounts in 1940 and 1941 and in considerable amounts in 1943

[1] Cooperative investigations between the United States Department of Agriculture and the Minnesota Agricultural Experiment Station. Paper No. 2114 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

Many of the collections of rusted oats were made by members of the barberry eradication organizations in various States. The following also contributed a number of valuable collections: D. G. Fletcher, of the Rust Prevention Association, Minneapolis, Minn.; I. M. Atkins, E. S. McFadden, H. C. Murphy, R. G. Shands, and T. R. Stanton, of the Bureau of Plant Industry, Agricultural Research Administration, U. S. Department of Agriculture; and T. E. Stoa, of the North Dakota Agricultural Experiment Station.

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(Table 1). Of the races isolated during the 5 years, only 8 and 10 attack Richland and its derivatives heavily (3).

In 1943 race 8 caused such conspicuous rust on the new rust-resistant varieties derived from Richland × Victoria crosses as to evoke independent comment from several plant pathologists, plant breeders, and men engaged in rust surveys. Although the severity of infection on the resistant varieties was relatively low in most cases, the infection type indicated complete susceptibility. That the rust actually was caused by race 8 is clear, as isolates of this race were obtained from Vicland, Boone, Tama, Control, from an unnamed line of Richland parentage, and from fields of unknown varieties. Race 8 always was isolated in pure form from the resistant varieties, but race 2 tended to predominate when both were isolated from collections of nonresistant varieties. In each of 5 such collections, the ratios between races 2 and 8 were 50:50, 85:15, 85:15, 80:20, and 50:50. These ratios are determined by estimating the number of type–1 and type–4 pustules on

TABLE 1.—*The prevalence of races of Puccinia graminis avenae in the United States from 1939 to 1943, inclusive*

Year	Race and percentage of total isolates								Total number of isolates
	2	5	7	8	10	12	2+5	8+10	
1939	55.7	41.6	1.2	1.2	0.3	0.3	97.3	1.5	251
1940	53.1	39.9	.....	2.4	3.8	0.7	93.0	6.2	286
1941	55.7	38.4	.....	1.6	3.3	1.0	94.1	4.9	305
1942	65.5	32.4	.....	0.4	1.7	.....	97.9	2.1	232
1943[a]	79.1	.....	.....	20.2	.....	0.7	79.1	20.2	421

[a] No distinction is made between races 2 and 5, nor between 8 and 10, in 1943.

Richland, produced by races 2 and 8, respectively, when differential varieties are inoculated in the greenhouse.

Race 8, originally found in Canada (2, 4), was collected in the United States for the first time in 1937, in Iowa, Wisconsin, and Pennsylvania. In 1938 it was found only in Virginia. During the first two years, therefore, it was found only in barberry-infested areas; and in 1939 it appeared in Ohio, Wisconsin, and Missouri. In 1940 it was found in Kansas and Texas, as well as in Iowa, Wisconsin, and Minnesota. It appears, therefore, to have become established first in the north and northeast, where barberries become rusted, and then to have extended southward and westward. Race 10 was first identified by Cotter (1) from rusted oats growing near barberries in Jefferson County, Wisconsin, under circumstances that make it virtually certain that the rust originated on the bushes. It appears, therefore, that both races 8 and 10 began their careers near barberries.

Race 8 then spread southward into Texas, where the uredial stage appears to have survived the winter of 1942–43; and the rust spread northward during the growing season of 1943. This sequence of events seems clear, although the evidence necessarily is circumstantial. Race 8 was isolated from rusted oats in Texas in the early spring of 1943 and was isolated subse-

quently from collections made in Colorado, Iowa, Kansas, Michigan, Minnesota, Missouri, Montana, Nebraska, North Dakota, Oklahoma, Pennsylvania, South Dakota, and Wisconsin (Fig. 1). Although inoculum of race 8 came from the South, it probably would not have been there except for barberries in the North. The fact that new or rare races have two possibilities of surviving the winter—uredial overwintering in the South and the telial-aecial sequence in the North and East—makes it doubly desirable to impede their possible production in the sexual stage on the barberry. When once produced, there is a possibility that they may perpetuate themselves in the uredial stage by traveling *via* air currents from north to south in the fall and from south to north in the spring and summer.

Despite their low prevalence, races 8 and 10 have been rather widely distributed. Even prior to 1943, race 8 was found in the following States during the period 1939–1942, inclusive: Iowa, Kansas, Minnesota, Missouri,

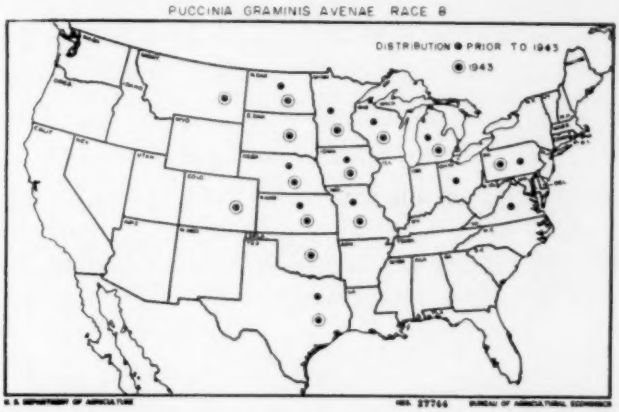

FIG. 1. Distribution of *Puccinia graminis avenae* race 8 in the United States.

Nebraska, North Dakota, South Dakota,[4] Ohio, Pennsylvania, Texas, and Wisconsin. During the same period, race 10 was found in Texas, Nebraska, Iowa, North Dakota, Minnesota, Wisconsin, Michigan, Virginia, and Pennsylvania.

There can be no certainty regarding the future of race 8 or other races to which Richland derivatives are susceptible. It is clear, however, that both 8 and 10 are rather widely distributed geographically and that there was considerable inoculum of race 8 during the summer of 1943. Given conditions favorable for extensive overwintering of the uredial stage in the South or for abundant development on barberry bushes in the North, or both, followed by favorable conditions for rust development during one or more growing seasons, this race might soon become strongly established. On the other hand, any one of several unfavorable circumstances might result in its subsidence. The tendency for the new rust-resistant oat varieties to reduce the prevalence of races 2 and 5 may favor the increase of race 8 and other similar races by eliminating competition. It is clear that there have

[4] Not included in figure 1.

been decided and important changes in prevalence of races of *Puccinia graminis tritici* (5, 6, 7, 8); and equally great changes may or may not occur in *P. graminis avenae*.

White Tartar, another of the differentials used in identifying races of *Puccinia graminis avenae* and also used as a stem-rust-resistant parent in crosses, is very resistant to races 2, 5, 8, and 10, but is susceptible to races 3, 4, 6, 7, 12, and 13. During the past 20 years these races either have not been found in the United States or have been so rare as to be of no consequence; however, during the past 5 years, 1939 to 1943, inclusive, race 7 was isolated from collections made in 1939 in barberry-infested areas in Wisconsin and Pennsylvania. It also was obtained from Oklahoma. In 1939 race 12 was isolated from aecial material collected in Pennsylvania, in 1940 it was found near barberries in the same State, in 1941 it was isolated 3 times from aecia from Pennsylvania, and in 1943 it was isolated from barberry areas in Pennsylvania and New York. The other races that attack White Tartar were not isolated in the United States, although races 1 to 10, inclusive, were found in Canada during the period 1925–1936, inclusive, according to Gordon and Welsh (2) and Margaret Newton (4).

SUMMARY AND CONCLUSIONS

The primary object of this paper is to call attention to the increase in prevalence in 1943 of race 8 of *Puccinia graminis avenae*. This increase may be only temporary or it may be relatively permanent, depending on conditions affecting the various phases of rust development in the near future.

The increase of race 8 in 1943 is important because it is evident that this race and race 10, which is combined with it for practical purposes, can cause heavy infection on Richland oats and on such varieties as Vicland, Boone, Tama, and others, mostly derived from Victoria × Richland crosses, and having the Richland type of resistance. Not only seedlings but also adult plants of these varieties are susceptible, confirming conclusions of Levine and Smith (3) regarding agreement between reaction of seedlings and adult plants of oats to physiologic races of *Puccinia graminis avenae*. As race 8 was isolated a number of times, especially in 1943, from large uredia on the hitherto generally resistant varieties, and as there was abundant rust on them in some localities, there is no question regarding their susceptibility. The real question is whether race 8 and the very closely related race 10 will increase, as certain races of *P. graminis tritici*, notably races 56 and 17, increased in recent years. Races 8 and 10 have been rather widely distributed geographically in the United States for several years, and the possibility of an increase in prevalence must, therefore, be recognized. Although a further increase in races 8 and 10 might jeopardize the varieties that derived their resistance from Richland, there are no present indications of an increase in the United States of those races to which White Tartar and its resistant derivatives are susceptible. Moreover, there are

indications from the work of Welsh (9) that it may be possible to obtain varieties combining resistance to all or most of the races now known.

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THE ANTAGONISM OF SOIL ORGANISMS TO FUSARIUM OXYSPORUM CUBENSE[1]

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(Accepted for publication October 6, 1943)

In the banana-producing sections of Jamaica it is common knowledge that some areas have a natural resistance to Panama disease, while other, near-by areas lack this resistance. Wardlaw (7) has pointed out that there are many unexplained aspects of the Panama-disease problem and refers to a situation on the lower Ulua River in the Republic of Honduras, where, notwithstanding periodic inundations by flood waters carrying débris from affected areas higher up, large areas have remained in a high state of production for a period of about 50 years.

An explanation of this natural resistance is suggested by experiments conducted by Alexopoulos, Arnett, and McIntosh (2) in 1938, which gave evidence of antibiosis between bacteria and fungi. Continuing this work Alexopoulos (1) tested out the ability of 80 Actinomyces species to inhibit the growth of *Colletotrichum gloeosporioides* on maltose agar. They were classified as 35 non-inhibitors, 31 weak inhibitors, and 14 strong inhibitors. Alexopoulos and Herrick (3) point out that actinomycetes differ in their ability to inhibit the growth of a given fungus in culture under the conditions of the experiment. Waksman, Horning, Welsch, and Woodruff (6) found that actinomycetes possessing antagonistic properties against bacteria and fungi were widely distributed in nature, especially in soils and composts. Two hundred and forty-four cultures were isolated at random from different soils; of these, 106 cultures possessed some antagonistic properties, and 49 were highly antagonistic.

An investigation into the character of this natural resistance of soils to Panama disease was, therefore, undertaken, resulting in the isolation of certain promising soil organisms (4). Most of the soil organisms encountered in this work have been actinomycetes (5), but careful taxonomic work has been deferred. These organisms fell into 3 classes: those (a) that apparently increased the growth of the Panama-disease fungus, (b) that did not interfere with the growth, and (c) that were antagonistic to the *Fusarium*.

MATERIALS AND METHODS

Soil samples were collected from various parishes in Jamaica, an effort being made to get soils of different composition and texture. Sixty-six samples were used that had been collected from St. Mary (45), St. James (13), St. Andrew (4), and St. Thomas (4).

A soil solution was made by shaking 20 g. of soil with water up to 1 liter. The mixture was filtered after about 30 minutes. This solution was used

[1] Acknowledgment is due the Jamaica Banana Producers' Association for support of this research.

with Parke, Davis and Co. granular agar for 2 per cent agar media. Each isolation and each test was made on the soil solution from which the soil organism was taken. The isolations were made by dilution methods with 0.00001 to 0.000001 g. of soil per plate.

The first tests were conducted in plates by making a line of the soil organism across the plate and introducing *Fusarium oxysporum cubense* about one-half inch from the soil culture. Later, a long, thin slant was made in test tubes and the same procedure followed. The soil organism was introduced by making a cut across the agar with a flattened needle, curved at the end, while the *Fusarium* was introduced about one-half inch above. Measurements of the mycelial growth of the *Fusarium* were made and recorded by the use of a low-powered microscope.

RESULTS

The soil organisms that exhibited antagonism to the Panama-disease fungus under the conditions of the experiment were placed in 3 classes:

1. Slightly antagonistic; organisms that retard the growth of *Fusarium oxysporum cubense.*
2. Antagonistic; organisms that have the ability to produce an area free from mycelium of *Fusarium oxysporum cubense.*
3. Very antagonistic; organisms that have pronounced ability to dissolve or inhibit the growth of *Fusarium oxysporum cubense.*

The isolations under observation from the 66 soil samples collected numbered 7642; 1020 of these were grown with the Panama-disease fungus. The antagonistic organisms found were classified as follows: (1) slightly antagonistic, 66; (2) antagonistic, 39; and (3) very antagonistic, 17 (Table 1). Only 15 of the soil samples produced very antagonistic organisms. There were 122 antagonistic organisms in all taken from the 66 soil samples; 17 samples produced no antagonistic organisms, 19 produced 1, 11 produced 2, 9 produced 3, 4 produced 4, 2 produced 5, 3 produced 6, and 1 produced 10. The antagonistic organisms were not evenly distributed, as was shown by the fact that 10 of the soil samples produced 54 antagonistic cultures, while 56 produced only 68.

A further experiment was conducted on Newry soil-solution agar to test out the ability of these antagonistic organisms to affect the growth of the Panama-disease fungus on soils other than the one from which they were taken. The 17 actinomycetes used were classified as very antagonistic (5), antagonistic (11), and slightly antagonistic (1). Eight of these organisms were antagonistic to *Fusarium oxysporum cubense* on the Newry soil-solution agar, while 9 did not exhibit the antagonism that they had shown on their own soil-solution agar. Of the 8 actinomycetes antagonistic to the *Fusarium* on Newry soil-solution agar 2 were listed as very antagonistic and 6 were listed as antagonistic on their own soil-solution agar.

From experiments conducted up to the present time it appears that the antagonism of actinomycetes varies with the soil solution, but the growth

TABLE 1.—*The effect of soil organisms on Fusarium oxysporum cubense growing on soil-solution agar*

Soil sample	Isolates	Number tested	Slightly antagonistic	Antagonistic	Very antagonistic
St. Mary					
1. Frontier	124	5	.....	.....	1
2. "	150	5	.....	.....	.....
3. "	182	5	.....	.....	.....
4. "	85	4	.....	.....	.....
5. "	87	4	.....	.....	.....
6. "	15	15	.....	.....	.....
7. "	29	29	1	.....	1
8. "	18	18	1	.....	.....
9. "	22	22	2	.....	.....
10. "	24	24	1	1	.....
11. "	15	9	.....	3	1
12. Gray's Inn	26	26	3	.....	1
13. " "	669	24	2	.....	.....
14. " "	550	21	1	.....	.....
15. " "	580	20	3	.....	.....
16. " "	27	23	4	6	.....
17. " "	114	17	1	1	.....
18. " "	326	23	1	1	.....
19. Newry	12	12	.....	.....	.....
20. "	266	20	.....	.....	1
21. "	38	15	3	.....	.....
22. "	25	10	.....	.....	2
23. Albion	15	15	.....	.....	.....
24. Clermont	15	15	.....	.....	1
25. Salem Manse	18	18	2	.....	.....
26. " "	160	20	.....	.....	.....
27. " "	54	20	2	.....	1
28. " "	81	20	1	.....	.....
29. Rosend	25	10	.....	.....	.....
30. "	43	15	.....	.....	1
31. "	69	10	.....	.....	1
32. Green Castle	65	20	.....	.....	.....
33. " "	127	20	.....	.....	.....
34. " "	14	10	1	.....	.....
35. Cape Clear	141	10	1	1	1
36. " "	75	20	5	.....	.....
37. " "	512	20	.....	.....	.....
38. " "	124	20	1	.....	.....
39. " "	55	10	2	1	.....
40. " "	618	20	1	.....	.....
41. " "	39	10	1	1	1
42. " "	282	20	.....	.....	.....
43. " "	107	10	1	.....	.....
44. " "	143	10	.....	.....	.....
45. Harmony Hall	161	16	1	.....	.....
St. James					
46. Guilsboro	5	5	.....	.....	.....
47. "	8	5	1	.....	.....
48. "	75	20	2	1	.....
49. "	142	16	5	1	.....
50. "	118	18	.....	1	.....
51. "	16	10	1	.....	.....
52. "	41	12	.....	.....	.....
53. "	7	7	2	2	.....
54. "	26	12	.....	.....	.....
55. "	136	20	.....	1	.....

TABLE 1.—(*Continued*)

Soil sample	Isolates	Number tested	Slightly antagonistic	Antagonistic	Very antagonistic
56. Guilsboro	111	15	3	2	.....
57. "	81	18	.....	4	2
58. Ducketts	46	20	1	1	.....
St. Thomas					
59. Amity Hall	64	20	4	2	.....
60. Holland	47	12	.....	1	.....
61. "	69	12	.....	1	1
62. "	74	12	1	.....	.....
St. Andrew					
63. Kirklands	27	22	3	.....	.....
64. "	44	12	.....	2	1
65. "	89	20	1	1	.....
66. "	89	22	.....	4	.....
Totals	7642	1020	66	39	17

of the Panama-disease fungus tends to be at about the same rate on all the soil solutions examined.

SUMMARY

Of the soil organisms, mostly actinomycetes, isolated from 66 soil samples in Jamaica, 122 exhibited antagonism to *Fusarium oxysporum cubense*. They were classified as 66 slightly antagonistic, 39 antagonistic, and 17 very antagonistic.

The antagonistic organisms were not evenly distributed in the soil samples, as 10 of the 66 samples had 44.2 per cent of the antagonistic organisms.

Actinomycetes antagonistic to *Fusarium oxysporum cubense* in their own soil-solution agar were not always antagonistic when tested in other soil-solution agar.

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XANTHOMONAS VIGNICOLA SP. NOV. PATHOGENIC ON COWPEAS AND BEANS

WALTER H. BURKHOLDER

(Accepted for publication January 1, 1944)

In August, 1942, A. A. Dunlap sent the writer specimens of cowpea plants (*Vigna sinensis* Endl.) showing large fissures and cankers on the stems, and later,[1] he published a description of the disease as it appears in the field under Texas conditions. The writer, without having seen naturally infected plants in the growing condition, made careful examinations of the stem lesions of these specimens and found that a great many bacteria were present in the necrotic tissue that evidently were the cause of the disease. Dilution plates from a number of the cankers gave rise to yellow bacterial colonies similar in growth characteristics to those of *Xanthomonas phaseoli* (E. F. Smith) Dowson. A search through literature, however, showed no report of this pathogen on the cowpea, and past experiences of the writer had shown that all attempts to infect this crop with the bean blight organism met with failure. No cowpea plants were available at the time of isolation with which to test the pathogenicity of these bacteria, but there were a number of young Red Kidney beans growing in the greenhouse. Inoculations on these plants, made by introducing the bacteria into a wounded stem, demonstrated that the organism was extremely virulent to this crop, and in approximately 3 weeks the plants were dead.

These results led to an investigation to determine the relationship of the cowpea pathogen to *Xanthomonas phaseoli,* and the ability of the two to infect beans, cowpeas, and related plants. The diseased cowpeas received from Texas were of the Chinese Red variety, so this variety was used in the experiments. Seed was kindly furnished by Dr. Dunlap. The bean variety used was the Red Kidney that is very susceptible to *X. phaseoli* and that had been shown to be very susceptible to the cowpea organism. In the inoculation experiments, 6 isolates of the cowpea pathogen, and 2 isolates of *X. phaseoli* were employed. The latter (XP4 and XP14) were 1 and 2 years old but still very pathogenic on beans. These 8 isolates proved pathogenic on the Red Kidney plants with approximately equal virulence. The symptoms were those always associated with *X. phaseoli.* It appeared in these experiments that the cowpea pathogen, after being in culture for 4 months, had lost its extreme virulence for the bean or that, during the second set of inoculations, conditions were not so favorable for virulent infection. The 6 cowpea isolates were pathogenic on the cowpea. Artificial infection of young cowpea plants by stem inoculation frequently led to their entire destruction without canker formation, but typical fissures resulted on the stem of older plants. Infection was not obtained on the cowpeas when inoculated with *X. phaseoli.*

[1] Dunlap, A. A. Two bacterial diseases in Texas. U. S. Dept. Agr., Bur. Pl. Ind. Plant Dis. Rptr., 27: 274. 1943.

Six months later these experiments were repeated in a similar manner with the exception that the bean blight pathogens were reisolates of those formerly used. By this time the cowpea pathogen had lost to a still greater extent its virulence for the bean. Small cankers appeared on the stem at the point of inoculation, and, occasionally, a leaf would wilt; but the plants did not die rapidly, as they did with recent isolates. The cowpea pathogen, however, still retained its virulence for its own host. No change was noted with the cultures of *Xanthomonas phaseoli.*

Further inoculation experiments were conducted on 3 other leguminous plants; alfalfa, lespedeza, and soy bean. These tests were duplicated but in no case was infection obtained. It, therefore, was evident that the cowpea organism was not any of the following bacteria that it resembles somewhat: *Xanthomonas alfalfae* (Riker *et al.*) Dowson, *X. lespedezae* (Ayers *et al.*) Burkholder, nor *X. phaseoli* var. *sojense* (Hedges) Starr and Burkholder.

The fact that recent isolates of the cowpea pathogen are extremely virulent on Red Kidney beans makes the organism of possible importance to the bean crop. Furthermore, the two pathogens being so similar in appearance, the cowpea organism already might be infecting the bean and isolates when made might be mistaken for *Xanthomonas phaseoli.* For these reasons investigations were undertaken to determine whether the two showed differences in cultural characteristics and biochemical reactions. If there were differences the identity of the pathogen then could be determined in culture.

Six isolates were used in describing the cowpea organism. These were selected from single colonies on dilution plates and originated from different lesions. Further dilution plates then were made to insure purity and each isolate was tested for pathogenicity. All behaved similarly, both pathogenically and in culture. Four isolates of *Xanthomonas phaseoli* (XP1, XP2, XP4 and XP14) were tested at the same time. The first two isolates were old cultures, but the last two were those used in the inoculation experiments reported above, and had been reisolated in January, 1943. A number of differences were found between the two pathogens, as is shown by the following description of the cowpea organism.

Morphology. The bacterium is a rod with rounded ends. In a 48-hour-old culture on beef-extract-peptone agar at 27° C., the size of cells are 1.76 μ (1.0 to 2.8 μ) by 0.7 μ (0.46 to 0.92 μ). The Congo red stain was used in this determination. The bacteria are motile with 1 polar flagellum. They are Gram-negative.

Cultural Characters. On beef-extract-peptone-agar slants at 27° C., a moderate growth develops along the streak in about 48 hours. It is filiform, with edges entire, glistening, primuline-yellow, butyrous. On potato-dextrose agar, growth is more abundant, mucoid, and pale-yellow to colorless. In beef-extract bouillon, a cloudy growth appears in 48 hours. There is a heavy yellow ring on the glass but no pellicle. In litmus milk after a week's growth there is a reduction of litmus and a light curd develops that soon becomes solid and remains so for several weeks. A slow peptonization follows and crystals, presumably tyrosine, are formed in the medium. At the end of 6 weeks the milk is a brownish-purple syrup. In shake cultures of beef-extract-peptone agar plus 0.5 per cent dextrose, colonies appear only on the surface of the medium, showing that the pathogen is a strict aerobe.

The optimum temperature for growth is 27° to 30° C. Maximum 37° C. and minimum between 6° and 9° C.

Biochemical Reactions. Growth in gelatin stabs is good; slight liquefaction begins on the second day and proceeds rapidly. Growth in tryptone broth is excellent and hydrogen sulphide production is evident after 4 days. The tests were made with strips of

filter paper impregnated with lead acetate. The Goré method shows no indole formation. There is a very slight to no growth in synthetic nitrate broth. Nitrites are not formed. In beef-extract-broth plus 0.1 per cent KNO_3 there is good growth but no nitrites at the end of 3, 5 and 10 days. $NH_4H_2PO_4$ can be utilized for nitrogen. There is no growth in Clara's medium[2] nor in the same solution when tyrosine is substituted for asparagine showing that these two amino acids cannot be utilized as a combined carbon and nitrogen source. However, in a richer medium tyrosine appears to be broken down, judging by the color reactions—a pink to a brown. On spirit-blue agar[3] there is a definite lipolytic action. In broth, growth is retarded with 2 per cent NaCl and inhibited with 3 per cent.

The cowpea pathogen grows slowly in a synthetic medium for carbohydrates[4] but after two weeks the following data were obtained. All carbon sources with the exception of the organic acids were filtered. Acid is formed in dextrose, galactose, lactose, maltose, sucrose, and raffinose. There is no growth in levulose, l-arabinose, xylose, rhamnose, glycerol, and salicin. An alkaline reaction is obtained with salts of citric and malic acid, while the growth in acetic acid was irregular. There is no growth in salts of lactic, formic, succinic, tartaric, and hippuric acid. Starch is hydrolyzed.

In the above experiments it was demonstrated that there are certain distinct differences between the two pathogens. Noticeably, the cowpea organism is much slower in growth in all media, and has a tendency to lose its viability in culture. This behavior cannot be accounted for on age of isolate, since XP4 and XP14 of *Xanthomonas phaseoli* were reisolates and they behave like the two older isolates of this species. The greatest difference between the two pathogens is noted in milk. The cowpea organism produces a solid curd that lasts several weeks before peptonization begins. The bean pathogen begins to clear the medium on the second day with no curd formation. The temperature relations also are different; *X. phaseoli* grows at 6° C. but not at 36° C., while the cowpea bacterium does not grow at 6° C., gives a slight growth at 9° C., produces a good growth at 36° C., but none at 38° C. There are some differences in the ability of the pathogens in their energy sources. *X. phaseoli* utilizes xylose, glycerol, succinic acid salts well, and levulose lightly; the cowpea pathogen does not utilize these carbon compounds, but utilizes mannitol, which *X. phaseoli* does not. The experiments from which the above data were collected were repeated with the same results.

Inasmuch as the cowpea pathogen differs definitely from *Xanthomonas phaseoli* in both pathogenicity and cultural reactions, it is considered a new species and the following name is proposed, *Xanthomonas* **vignicola** sp. n.

SUMMARY

A description is given of *Xanthomonas* **vignicola** n. sp., a bacterium that causes cankers on the stems of cowpeas and a blight of the common bean. The pathogen is similar to *X. phaseoli* but differs distinctly in its pathogenicity and in certain cultural characteristics.

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[2] Clara, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. New York (Cornell) Agr. Exp. Stat. Mem. 159: 1–34. 1934.

[3] Starr, Mortimer P. Spirit blue agar; a medium for detection of lipolytic microorganisms. Science 93: 333–334. 1941.

[4] Society of American Bacteriologists, Committee on Bacteriological Technique. Manual of methods for the pure culture study of bacteria. (Geneva, N. Y.) 1923. Leaflet II, ed. 8, 1942, p. 15.

CHARCOAL ROT OF IRISH POTATOES[1, 2]

R. D. WATSON

(Accepted for publication November 17, 1943)

The rotting of Irish potatoes has been a serious disease in East Texas to the knowledge of more than a generation of growers. Most of the rotting occurs in the field after the plants reach maturity and in the early part of the storage period. The primary causal agent appears to be *Sclerotium bataticola* Taub. This fungus has been reported[3] as causing decay of Irish potatoes in the Eastern Mediterranean area and in India. A serious tuber decay of Irish potatoes grown under irrigation in the Texas Panhandle in the summer of 1943 was later found to be due to this same fungus.[4]

All varieties of Irish potatoes grown in this region appear to be susceptible to charcoal rot. The varieties on which it was observed this year were Bliss Triumph, Irish Cobbler, Katahdin, Chippewa, and Rural. Losses often have amounted to 50 per cent or more of the crops and seldom are they below 5 per cent, with an estimated average of 15 to 20 per cent of the stored crop in 1943. The amount of decay is closely correlated with the date of digging and the moisture content of the soil, particularly at digging time. Wet soils and high temperatures favor the development of the disease.

Charcoal rot attacks the maturing plant at the onset of hot weather. It probably infects the seed piece, develops in the plant, and goes down through the stolon to cause the conspicuous rot on the tuber. Early-, medium-, and long-season potatoes as well as early- and late-planted ones died all at about the same time, even though the soil moisture seemed adequate for their growth. This may indicate that this is an important disease of the plant, as well as of the tuber.

The external symptoms on the tuber are typically a blackened and somewhat soft or semi-wilted infected area. The internal symptoms show blackening to various depths, but ordinarily the discoloration is restricted to the outer area of the tuber. The infections are most often at the stolon end and around the eyes (see Fig. 1). During wet weather swollen lenticels also provide points of entrance by the fungus. *Sclerotium bataticola* inoculated into the tuber produced the typical symptoms of the rot whether the isolate came from potato or from cowpeas. The fungus was reisolated from these inoculated tubers.

The later and final stages of the rot are ordinarily associated with one or both of the following organisms: (1) *Erwinia carotovora* (Jones) Holland in which case the tuber breaks down into a typical soft rot but with the

[1] Approved by the Director of the Texas Agricultural Experiment Station as Technical Paper No. 821.

[2] Previous mention of this disease has been made, (Abstract) Phytopath. 33: 1120. (1943) 1944.

[3] Rev. of Appl. Mycol. 9: 561; 10: 437; 11: 126; 17: 552; 19: 198.

[4] A survey of Irish potato diseases in Texas Panhandle fields in August, 1943. Texas Agr. Exp. Stat. Mimeographed Prog. Rept. No. 859.

blackening of the original charcoal rot still evident. This organism was most commonly associated with charcoal rot at digging time or shortly afterwards. (2) A *Fusarium* sp., which with *S. bataticola* produces a water-soaked, spongy rot, and the tuber ordinarily dries into a mummy with a white fungus outgrowth at the eyes or injured portions. This was more common later in storage. The rotting that occurs in storage seems to a large

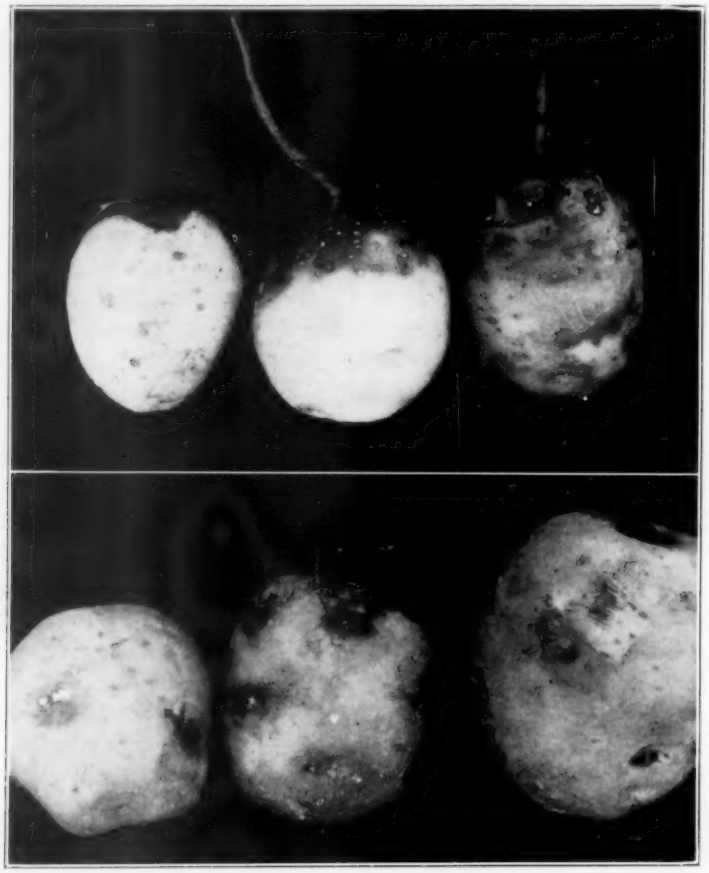

FIG. 1. Above, typical stolon infection of the charcoal-rot fungus on the tubers of Irish potato. Below, eye infection by the charcoal-rot fungus, *S. bataticola*.

extent attributable to infection in the field. Charcoal rot on the tuber somewhat resembles late-blight rot, but the two are disinguishable to one familiar with the latter.

High temperature seems the most important of the environmental factors affecting incidence and severity of charcoal rot. Abundant moisture also favors development of the disease. Although *Sclerotium bataticola* is pathogenic at temperatures below 80 to 85° F., the rotting is slow, and, as the temperature rises, the rate of rotting is very much accelerated. The disease has

been reported on Irish potatoes for the most part from tropical or semi-tropical regions where high soil temperatures during harvest season might be expected. In East Texas the crop is planted early in the spring and matures as summer begins. Undoubtedly the high soil temperatures of summer are responsible for its common and destructive occurrences.

Control recommendations are based on the present knowledge of the disease and its chief epiphytological factors. Since the organism is present in most of the soils in the region and has a wide host range, rotation alone will be of little value. It is important to remove the tuber from the ground before it becomes infected and before high summer temperatures increase the activity of the organism. The use of early varieties, planted and harvested early, aids in avoiding field infection of the tubers. Care must be used in handling these immature tubers to avoid bruises, sunscald, and other injuries that will increase the amount of rot. Only well-drained land should be used. Potatoes should be sorted to remove any early-stage rot and kept dry in crates, which provide for air circulation. Forced circulation will reduce the secondary rots. Cold temperatures of 34 to 38° F. reduce the amount and rate of rot during storage.

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PHYTOPATHOLOGICAL NOTES

Guttation-salt Injury on Leaves of Cantaloupe, Pepper, and Onion.—The formation of salt deposits on the leaves of various plants following guttation, and injuries associated with these deposits, have been observed[1] in the Winter Garden Region of Texas. As early as 1804, De Saussure[2] reported as follows on a certain "maladie blanche" apparently well recognized at that time as a nonparasitic disease on cucurbits and other garden plants:

> . . . the superfluous salts accumulate in certain plants upon the surface of their leaves, and form an incrustation which makes them perish, by preventing transpiration. Such is in large part the origin of the white malady ('maladie blanche'), which attacks the Cucurbitaceae and many garden plants. This malady begins, in the case of the cucurbits, with the viscous drops, which appear principally upon the surface of the leaves in the vicinity of the petiole. These drops dry out and form white conspicuous powdery stains, which extend and multiply successively near the circumference of the leaf. I have removed this incrustation; it is not soluble except in part, in water and in alcohol. These solutions after evaporation and drying presented a deliquescent salt, which had all the properties of calcium muriate united with an inappreciable quantity of magnesia; it was abundantly precipitated by silver nitrate, potassium oxalate, and the alkaline carbonates, but not at all by baryta water, and it was almost unalterable by fire. The saline and the earthy metals' part of the incrustation formed about one-third of its weight; it is enveloped by a white vegetative substance insoluble in water and in alcohol, and sufficiently abundant because the incrustation of the same was not sensitively attracted by the humidity. This malady attacks principally the old plants which grow upon a soil highly saturated with animal manure, and under layers where the leaves have not been washed by the rains.

On the edges of cantaloupe leaves, local necrosis, in the form of small brown semicircular areas, has been observed to develop at the hydathodes following guttation and accumulation of salt deposits at these points. These necrotic spots are frequently bordered, and at times preceded, by a chlorotic area (Fig. 1, A). The spots may coalesce and form a band of necrotic tissue around the entire margin of the leaf. In some cases the whole leaf may die. Cantaloupes may guttate frequently and profusely, but, if the concentration of the salts in the guttation water be low and no large amounts of salts be deposited on the leaf, no injury may occur. The composition of these deposits is not entirely known, but they seem to contain several common soluble salts in addition to calcium carbonate. Similar injury has resulted, both in greenhouse and field, under partly controlled conditions by inducing guttation and heavy salt deposits or by placing guttated salts from Tamarix in the droplets on the cantaloupe leaves.

With the pepper plant, salt deposits have been observed on the surface of the leaves following guttation through the upper surfaces, accompanied by injury in the form of large necrotic areas, often with a water-soaked border (Fig. 1, B and C). This injury has been reproduced in the laboratory by transferring salts from the surface of the necrotic areas to uninjured

[1] Ivanoff, S. S. Chemical deposits on foliage of citrus and other plants and their possible relation to chlorosis and yield. Texas Agr. Exp. Stat. 54th Ann. Rpt., 181–182. 1941.

[2] De Saussure, Théodore. Recherches chimiques sur la végétation. p. 264–265. Paris. 1804. (The quotation given here is a translation from the original French, by the writer.)

pepper leaves in moist chambers. The salts from peppers were very hygroscopic and seemed to contain little or no calcium carbonate.

On onions, amorphous or crystalline white deposits in the form of small aggregates or lumps up to about 1 mm. in diameter have been observed, from few to several hundreds on a single leaf (Fig. 1, D). Some time following their formation, there usually appear specks or spots underneath the deposits. These may be white, pale yellow, or tan, apparently depending on their age. Aggregates of these spots resemble the leaf symptoms of "onion blight," a nonparasitic trouble of economic importance.[3] Similar symptoms, including blighting of onion leaf tips, have been produced experimentally by placing dry guttation salts collected from Tamarix on the young onion leaves, where the salts deliqueseed.

Since guttation seems to be a common phenomenon, it is possible that other plants may be subject to this same injury. Possibly, the nonparasitic

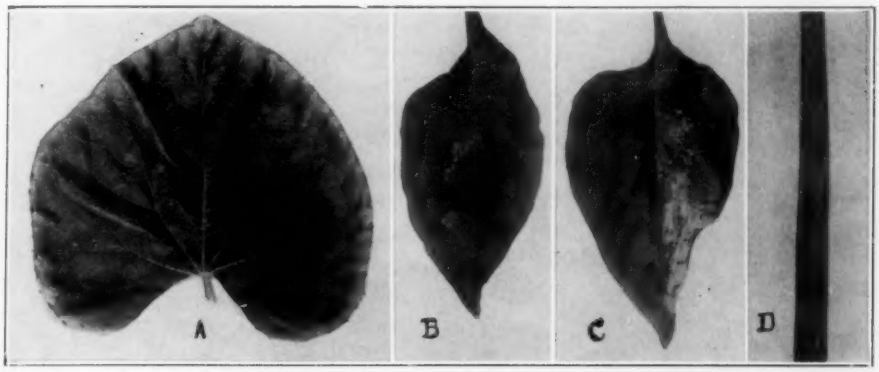

FIG. 1. Guttation-salt deposits and associated injury to leaf tissues. A. Cantaloupe leaf showing numerous semicircular necrotic lesions at the hydathodes (where guttation-salt deposits were previously observed) with nearby chlorotic and necrotic areas. B. Pepper leaf with dry salt deposits following guttation through the upper surface. C. Pepper leaf with guttation-salt injury at the moistened lower portion of the leaf following partial dissolving of salt deposits. D. Onion leaf with salt deposits, beneath which were formed small spots of dead tissue.

tipburns of lettuce, potato, and of other plants sould be re-examined from this standpoint.—S. S. IVANOFF, Texas Agricultural Experiment Station, Substation 19, Winter Haven, Texas.

Sporonema Rot of Apples.—In March, 1936, a York Imperial apple that had been stored at 36° F., was found to have a large decayed area, with numerous pycnidia in the center. The causal organism was identified by C. L. Shear as *Sporonema oxycocci*, a fungus causing decay of stored cranberries and first described by him in 1907.[1] This fungus never has been reported as causing decay of apples, and no further cases of natural infection have been observed. As cranberries were stored in the same room with

[3] Ivanoff, S. S. Onion "blight." Texas Agr. Exp. Stat. 51st Ann. Rpt. 260–261. 1938.

[1] Shear, C. L. New species of fungi. Bul. Torr. Bot. Club 34: 305–317. 1907.

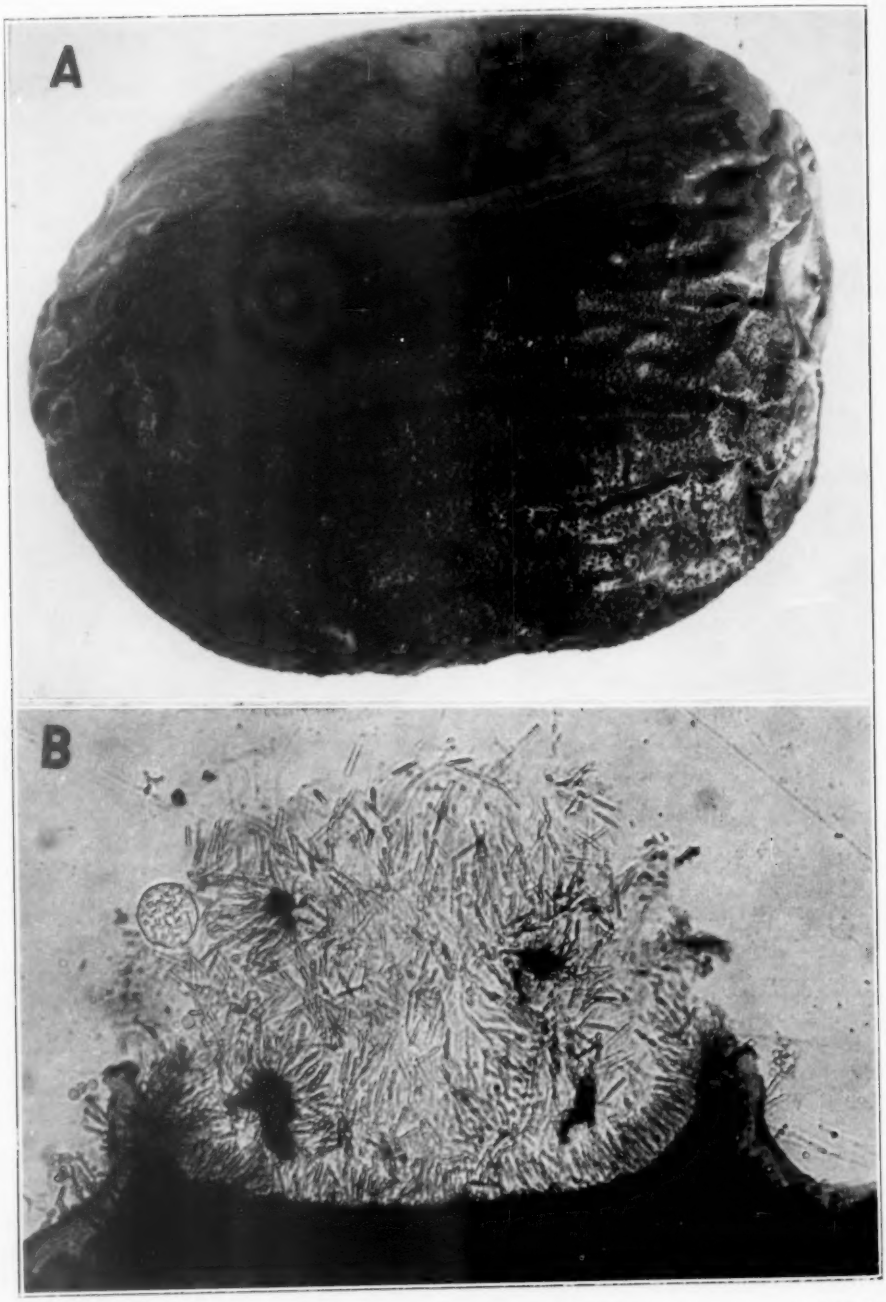

FIG. 1. *Sporonema oxycocci* causing artificially produced rot on York Imperial apple at 50° F. A. Numerous pycnidia protruding through the surface of the decayed tissue. B. Cross section of a pycnidium showing the cylindrical spores.

the apples, the decay was noted with interest, but considered as incidental. However, in the course of other studies on the source of certain fungi causing decay of stored apples, a species of *Sporonema* was isolated and was again found to cause decay when inoculated into apples. This experience made it seem worth while to report the susceptibility of stored apples to *Sporonema* and to include briefly certain observations and descriptions.

This fungus was found causing decay in 6 of the 44 inoculations, where small quantities of surface soil taken from a wooded area near an apple orchard were inserted into oblique surface wounds on apples and these were then stored at 31° F. for development.

The disease was produced artificially; typical lesions developed in York Imperial apples that were slightly wounded and dipped in an aqueous pycnospore suspension, and subsequently stored at 31°, 36°, and 50° F. In these tests large, open lenticels also occasionally served as infection courts.

The rot developed slowly at 31° F., but in the course of several months of storage the lesions developed to serious proportions (Fig. 1–A). The decayed tissue was pale-brown at first, but became progressively darker with age until, at the time the pycnidia (Fig. 1–A) were formed, the underlying tissue was quite black. The rot that developed at 50° F. was darker than that at 31° F. Mature pycnidia have not been found at 31° F., but have occurred on apples at 36° F. The pycnidia originate immediately below the decayed peel and become erumpent through it (Fig. 1–B).

The fungus grew readily on Thaxter agar at a wide range of temperatures. After 24 days the average diameters of the colonies as grown at various temperatures on this medium were: 32° F. = 5.8 mm.; 41° F. = 27.6 mm.; 50° F. = 44.0 mm.; 59° F. = 50.8 mm.; 68° F. = 64.8 mm.; 77° F. = 79.5 mm.—L. P. McCOLLOCH, Bureau of Plant Industry Station, Beltsville, Md.

Bacterial Canker of Cowpeas.—The latter part of July, 1942, a grower in Pottawatomie County, Oklahoma, sent to the agricultural experiment station at Stillwater some diseased Whippoorwill cowpea plants for diagnosis. Stems of the diseased plants were characterized by prominent, swollen, cracked cankers. These cankers were located anywhere from the ground level to a height of 6 or 8 inches. Dilution plates from these cankers yielded abundant bacterial colonies and an occasional colony of *Fusarium* sp. A survey of the literature gave no clue as to the nature of the trouble.

In early August, 1942, it was possible to make a personal inspection of the field. The crop was almost a total loss. Only after considerable search was it possible to find what could be termed a healthy plant. In many cases it was noticed that diseased plants had a tendency to break over at the cankered area.

In October, 1942, the same trouble was observed to be present in a mild form in a planting of Chinese Red cowpeas on the Experiment Station plots at Stillwater. The farm foreman stated that the disease had been present in small amounts each year for at least 7 years.

In the 1943 growing season the disease was again noticed at Stillwater and Perkins by early July. By August several varieties in the cowpea nursery at Perkins were badly diseased (Fig. 1). A count of the diseased plants in each 100-foot row gave the data contained in table 1. From this table it is evident that the varieties Wood's Sumptuous, Columbia, Whippoorwill, Early Red, and a few others are susceptible to this disease, while such varieties as Buff or Iron, Victor, Potomac or Calico, Speckled Crowder, Brown Crowder, and several others are either resistant or escaped infection.

During the 1943 growing season the disease has also been observed at other points in Payne County and in Creek, Comanche, and Stevens Coun-

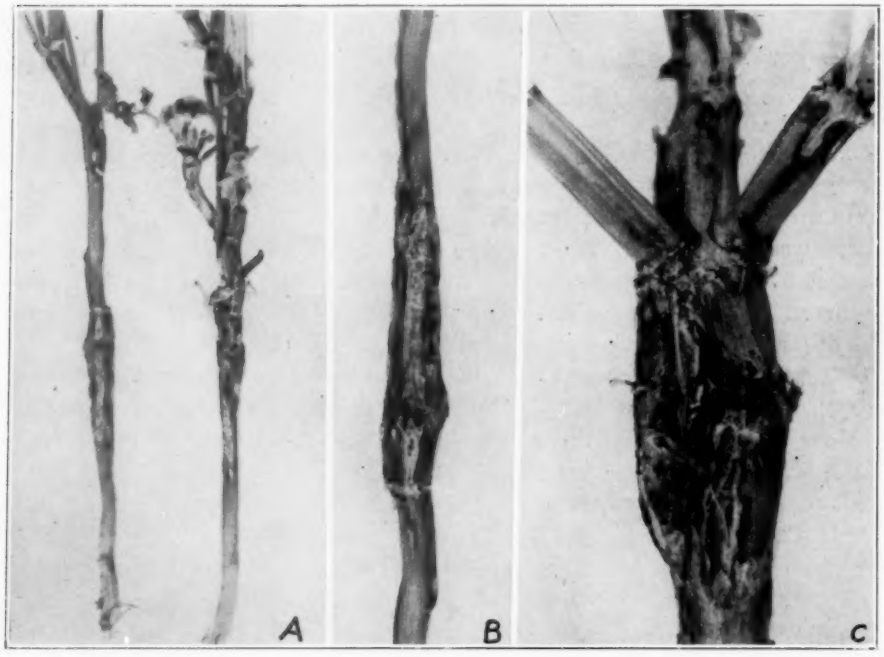

FIG. 1. Bacterial canker of cowpeas. A. Two plants showing the location of the cankers. B. Close up of a typical canker. C. Close up of a badly cankered plant showing the formation of secondary cankers on the branches.

ties, Oklahoma, and near Stuttgart, Arkansas. The disease also appears to have been recently observed in Texas.[1]

According to the unpublished thesis of E. W. Brillhart, in the Botany and Plant Pathology Department at Oklahoma Agricultural and Mechanical College[2] the first authentic record of this disease in Oklahoma is that reported by him from Perkins in 1931. The description and photographs in the thesis leave no doubt but that it is the same canker-producing disease that has been observed during the past two summers. Brillhart states that it may attack leaves, stems, pods, and seeds, but that the most conspicuous

[1] Dunlap, A. A. Some notes on newly observed or unusual diseases. U. S. Dept. Agr. Plant Dis. Rptr. 27: 274. 1943.

[2] Brillhart, E. W. Bacterial canker of cowpeas. An unpublished thesis. Okla. A. and M. College. 1934.

symptom is the stem canker. He indicates that the causal organism is *Bacterium vignae,* which he isolated from diseased leaves, seed, and cankers. He even isolated the bacterium from cankers that had overwintered in the field. Thus it would appear that the organism may overwinter in both the seed and the soil. Brillhart was able to produce typical leaf symptoms in the greenhouse with bacteria isolated from leaves, seed, and cankers of dis-

TABLE 1.—*Bacterial canker of cowpeas. Number of diseased plants per three 100-foot row replicates. Perkins, Oklahoma, 1943*

Variety	Replication			Total
1. Wood's Sumptuous	235	433	152	820
2. Columbia	183	39	38	260
3. Early Red	90	98	33	221
4. Whippoorwill	88	31	102	221
5. Dixie Queen	36	68	70	174
6. Early Ramshorn Blackeye	86	17	38	141
7. Giant Resistant Ramshorn Blackeye	9	65	53	127
8. Clay	4	59	28	71
9. Arlington	8	27	22	57
10. Large Virginia Blackeye	2	29	17	48
11. Chinese Red	4	11	29	44
12. Holstein	15	10	13	38
13. Cream or White Crowder	6	9	16	31
14. Blacks	6	7	17	30
15. Red Ripper	5	2	14	21
16. Virginia Blackeye	0	11	10	21
17. Blue Goose	10	4	5	19
18. Rice or Lady	16	1	2	19
19. Lady Edible Cowpea	2	4	9	15
20. Groit	2	0	5	7
21. Brown Crowder	1	0	3	4
22. New Era	0	3	1	4
23. Braham	0	0	3	3
24. White Browneye Crowder	0	0	3	3
25. Brown or Sugar Crowder	0	0	2	2
26. Potomac or Calico	0	0	1	1
27. Speckled Crowder	0	1	0	1
28. Buff or Iron	0	0	0	0
29. Victor	0	0	0	0

eased plants. The bacterium was reisolated from these inoculated plants. However, he does not specifically state that he was able to reproduce the cankers under greenhouse conditions.—DONALD E. HOFFMASTER, Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma.

Bibliography and Nomenclature of Puccinia oryzae.—This fungus, apparently a distinct race of *Puccinia graminis* P., is known only from limited areas in Spain and Italy[1] where scant attention has been accorded it because under the peculiar ecological conditions and crop practices there encountered it is ordinarily not an economic menace. It has, however, such destructive potentialities that plant quarantine measures against it elsewhere are abundantly justified. The chief references in scientific literature to this pathogen are unfortunately conspicuous only for their

[1] It is significant that what is doubtless this same fungus has been recorded also from Roumania by O. Savulesco (p. 305): Micromycètes trouvés sur le riz en Roumania—Acad. Roumaine, Bull. Sect. Sci. 22 (no. 7): 305–308. 1940.

bibliographic shortcomings. They are commented upon in chronological order, quoting the nomenclature employed in each instance as follows:

1903. Briosi, G. Rassegna crittogamica pel primo semestre 1903—in Bollet. Uffi. Minist. d'Agr., Ind. e Comm. N.S. Anno II, 4: 83–95 fasc. 203 (Rome, Italy).—Briosi on pages 84–85 discusses the symptoms of the disease and the characteristics of the pathogen, which he calls "*Puccinia oryzae*" without, however, labeling it as a newly named species, omitting a technical description, yet, without reporting any previous observations of it. This particular contribution of Briosi's was probably printed and distributed as a separate and it has most likely been confused, as will be pointed out further on, with another paper having a similar title, which did not contain any reference to his *Puccinia oryzae*, namely: "Rassegna crittogamica pel primo semestre 1903" in Att. Ist. Bot. Pavia 2 ser. 9: 323–339, (Milano) 1911.

1908. Trotter, A. Flora Italica cryptogamica Pt. 1, fasc. 4 (Uredinales), pp. 519.—On p. 477 Trotter notes a "*Puccinia oryzae*" upon rice as "Riso in Lombardia (Bibl. 263 1 sem. an. 1903)." He did not cite any author for the species, although by his reference it should be Briosi. The citation involves difficulties since by "Bibl. 263" is meant no. 263 of Traverso's Bibliography in Fl. Ital. Crypt. Pt. IV, fasc. 1, which is: "263) 1886–1903—Briosi, G.—Rassegna crittogamiche. Milano (in At. Ist. Bot. Pavia vol. I–VIII in 8°)." The article by Briosi for "1 sem. an. 1903" does not, however, appear in vols. I–VIII as indicated by Trotter but as cited above in Att. Ist. Bot. Pavia vol. 9, 1911. Although in the main duplicating information contained in the 1903 report by Briosi first cited above, it omits entirely any mention of *Puccinia oryzae* or of *Puccina graminis* upon *Oryza*, showing that Trotter failed to realize that the two papers by Briosi were not identical.

1909. Sydow, P. & H. Monographia Uredinearum 1: pp. 972.—This monograph on p. 695 records *Oryza sativa* as one of the hosts for *Puccinia graminis* P. but does not note *P. oryzae* or any special rice form or variety of the parasite, nor does it cite Briosi's account.

1915. Florensa y Condal, Jose. Sindicato de Riegos del Delta del Ebro, la Infermedad del Arroz, Terragona pp. 32, ill. (abstr. in Mo. Bull. Agr. Intell. & Pl. Dis. Int. Inst. Agr. (Rome) 6: 460–470, 1915 no. 3.) (abstr. in Techn. Rundschau 7: 514–515, 1915).—This is a semi-popular account of the rust fungus here named *Puccinia oryzae* (author not noted) in connection with a seriously diseased condition of rice in a rice-growing area of Spain.

1918. Gonzales Fragoso, R. Enumeraçion y distribuçion de las Uredales . . . Iberica e Islas Baleares. Trabajos Mus. Nac. Cienc. Nat. Ser. Bot. Num. 15: 5–267.—The fungus is here referred to (p. 39) as "*Puccinia graminis* P. f. *oryzae* (Risso) in Rass. critt. Padova 1 sem. an. 1903—Trotter, Ured. de la fl. ital. p. 477." The author had doubtless misunderstood the meaning of "riso," the Italian word for rice, in Briosi's account, possibly confusing it with the name "Risso" which he might have taken to be a reference to Antoine Risso (1777–1845) a botanist of an earlier period who appears to have had no connection whatever with the name of this fungus on rice. It is obvious that he also confused Padua with Pavia.

1919. Oudemans, C. A. J. A. Enumeratio systematica Fungorum 1: pp. 1230.—On p. 723 *Puccinia graminis* P. is recorded as upon *Oryza sativa*, citing in addition to the Sydow Monographia (l.c.) Persoon's "Disp. meth." 1797 and Persoon's "Synops." 1801, although in neither of Persoon's compendia is there any reference to *Oryza* as a host for the species.

1924. Gonzales Fragoso, R. Flora Iberica Uredales, pp. 424.—The rice fungus is here (p. 26) considered under the name "*Puccinia graminis* P." without further elaboration.

1882–1931. Saccardo, P. A., *et al.*—Sylloge Fungorum vols. 1–25. A reasonably diligent search of the indexes to these 25 volumes has failed to reveal there any citation to this fungus as upon *Oryza*, unless so noted under the abbreviation "etc."

The name *Puccinia oryzae* having no technical description is clearly a nomen nudum, but as treated by Gonzales Fragoso (l.c., 1918) it would be valid as *Puccinia graminis* P. f. *oryzae* Frag.—WILLIAM W. DIEHL, Bureau of Plant Industry Station, Beltsville, Md.